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ENVIRONMENTAL BEHAVIOR AND FATE OF EXPLOSIVES IN GROUNDWATER FROM THE MILAN ARMY AMMUNITION PLANT IN AQUATIC AND WETLAND PLANTS

FATE OF TNT AND RDX

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Environmental Behavior and Fate of Explosives in Groundwater from the Milan Army Ammunition Plant in Aquatic and Wetland Plants

Fate of TNT and RDX

by

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Abstract

The present study was performed to elucidate the environmental behavior and fate of TNT and RDX in aquatic and wetland plants collected from a field-scale wetland demonstration deployed at Milan Army Ammunition Plant for removal of explosives from groundwater. The study had three objectives: (1) To establish the physiological capacity of plants to absorb and transport TNT or RDX from explosives-contaminated groundwater in the absence of substrates and their sorptive activities; (2) To quantify partitioning of TNT and RDX between plant portions; and (3) To establish the short-term chemical fate of TNT and RDX in plant tissues of these species. Substrates in which these plants were rooted at the Milan field site (sediment, gravel) were also incubated without plants to investigate sorptive activities, and to evaluate microbial/chemical transformation of TNT and RDX that may affect the explosives availability to plants.

Hydroponic batch incubations of plant or substrate treatments with ^{14}C-TNT or ^{14}C-RDX were used to evaluate explosives fate. The study surveyed seven plant species and two substrates in sequential, independent incubations of 7 and 13 days with TNT and RDX, respectively. Radiolabel distribution in intact plants was followed using autoradiography and radio-analytic imaging. Parent compounds and degradation products were determined through chemical (HPLC) analyses of plant tissue extracts, aqueous phases and substrate extracts. The fate of radiolabel in plants and substrates was followed using thin layer chromatography and radioanalytic imaging.

While growth of most plants except parrot-feather was low in groundwater amended to contain 1.6 to 3.4 mg TNT L^{-1}, TNT disappeared completely from groundwater incubated with plants in 7 days. Highest specific removal rates were found in submersed plants in elodea (0.05 mg TNT g FW^{-1} d^{-1}) and in emergent plants in parrot-feather, sweet-flag, and reed canary grass (0.006 mg TNT g total FW^{-1} d^{-1}). TNT declined less with substrates, and least in unplanted controls. Radiolabel was present in all plants after incubation. In the submersed species radioactivity was concentrated in physiologically active roots and shoots, and in emergent species in roots. Mineralization to CO_2 was very low, and evolution into volatile organic compounds was negligible. TNT residues were extremely low or below chemical detection in plant tissues. Radioactive degradation products accumulated at the sites of uptake and transport was limited. TNT degradation took place via reduction of a single nitro-group. At least five other unknown metabolites were found.

In RDX incubations growth of submersed plants was normal, but growth of emergent plants was reduced in groundwater amended to contain 1.5 mg RDX L^{-1}. RDX disappeared less rapidly than TNT from the incubated groundwater. Highest specific RDX removal rates were

found in submersed plants in elodea (0.004 mg RDX g FW^{-1} d^{-1}), and in emergent plants in reed canary grass (0.001 mg RDX g total FW^{-1} d^{-1}). Radiolabel was present in all plants after incubation. Mineralization to CO_2 was low, but relatively higher than in the TNT incubation. Evolution into volatile organic compounds was negligible. Radioactive degradation products accumulated at physiologically active sites, and transport to leaves was substantial, ranging from 23% of total plant radioactivity in sweet-flag to 81% in parrot-feather. RDX residues were low in most plants, or below detection in the below-ground portions of two emergent species. The RDX residues ranged from 0.3 µg g FW^{-1} in pondweed to 8.6 µg g FW^{-1} in parrot-feather shoots. RDX degradation into at least five unknown compounds was shown to occur.

No detectable residues of either explosive were found in substrates.

The promise of phytoremediation in constructed wetlands as a technology for removal of explosives from groundwater is supported by several results of this study. 1) The rapid decrease in TNT and relatively slower decrease in RDX in the presence of certain aquatic or wetland plants under viable environmental conditions, 2) The relatively rapid metabolism of the parent compounds inside the plants, and 3) Low explosives residues in plant tissues and substrates. However, it must be realized that metabolic pathways of degradation of TNT and RDX in plants are still unknown, and that certain explosives degradation products may exert other biological and toxicological activities. Decreases in TNT and RDX levels in water with plants may also be due partly to chemical binding between explosives transformation products and organic matter. The generation of plant-specific dissolved organic matter and leachates, may also play a role in stimulating microbial activity and result in degradation of explosives.

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Preface

The work reported here was conducted as part of the Army Environmental Center's "Phytoremediation of Explosives-Contaminated Groundwater Using Constructed Wetlands" project under a partnering agreement involving the US Army Environmental Center (AEC), Aberdeen Proving Ground, MD, as the lead agency with Ms. Darlene F. Bader as project manager, and the US Army Waterways Experiment Station (WES), Vicksburg, MS, and the Tennessee Valley Authority (TVA), Muscle Shoals, AL, providing technical support. Funding was provided under the DoD's Environmental Security Technology Certification Program (ESTCP).

This study was conducted at WES under the general supervision of Dr. John W. Keeley, Acting Director, Environmental Laboratory (EL), and under the direct supervision of Dr. Richard E. Price, Chief Environmental Processes and Effects Division (EPED).

Technical help was provided by Ms. Linda Nelson and Mr. Robbie B. Godwin, Ecosystem Processes and Effects Branch (EPEB), and Ms. Anne B. Stewart, AScI Corporation, McLean, VA. HPLC analysis of explosives and TNT degradation products in water and plant extracts, and chemical characterization of the groundwater was performed by the Environmental Chemistry Branch, Environmental Engineering Division (EED), EL.

The report was prepared by Dr. Elly P. H. Best of the AScI Corporation, with contributions from Drs. Herb L. Fredrickson and Susan L. Sprecher (EPED), and Dr. Steven Larson (EED). Valuable discussions and comments during the course of the study were provided by Drs. Judith Pennington and Bill Davis, EPED. Technical reviews were provided by Drs. Pennington and John Madsen.

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1- Introduction

Explosives and Phytoremediation

Munitions material such as 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and their combustion and degradation products can enter the environment from production activities and field usage and disposal (Small and Rosenblatt 1974; Spanggord et al. 1980). The presence of these substances is of concern because of their potential toxicity and mutagenicity (Marvin-Sikkema and De Bont 1994).

The utilization of plants for clean-up of the environment has received relatively little attention despite the fact that plants, like microorganisms, play an important role in nature in sustaining and restoring environments. The capabilities of plants to absorb, accumulate and metabolize, directly or indirectly, various organic substances suggests their utilization in the remediation of contaminated environments in a technology named phytoremediation (Salt et al. 1995; Schnoor et al. 1995).

In the aquatic environment, both TNT and RDX can disappear rapidly from water due to photolysis (Spanggord et al. 1980; Gorontzy et al. 1994). Furthermore, adsorption of these explosives to sediment is not significant (Spanggord et al. 1980). Relatively rapid rates of TNT transformation by microorganisms have been reported (Spanggord et al. 1980; Gorontzy et al. 1994), but slower rates of RDX -- the latter predominantly under anaerobic conditions (Binks, Nicklin and Bruce 1995; Sikora et al. 1997). Recently, TNT was found to disappear rapidly from water in the presence of several algae and submersed and emergent plants, while RDX decreased far more slowly (Schnoor et al. 1995; TVA 1995; Best and Sprecher 1996; Best et al. 1997a; Best et al. 1997b; Best et al. 1997c; Best et al. 1997d). The decrease in RDX concentration was largely attributed to plant-stimulated activity of microorganisms inherent to the explosives-contaminated water. Recent flow-through studies of 2-month duration, however, suggested that, in the presence of some aquatic and wetland plants, the initial rapid disappearance of TNT is largely due to adsorption processes, followed by some plant-leachate stimulation of microbial degradation. Disappearance of RDX was gradual and slower, but the actual RDX removal rates were twice as high as for TNT (Best et al. 1997a). Degradation of TNT by freshwater sediments has been shown to be mediated by enzymes of aquatic plant origin (Van Beelen and Burris 1995).

Biotransformation depends on the activity of the organisms involved, and can be decreased by toxic compounds, such as explosives. Both TNT and RDX have been shown to be toxic to aquatic plants at concentrations of ≥ 2 mg L^{-1}, depending on the species (Schott and Worthley 1974; Smock, Stoneburner and Clark 1976; Best et al. 1997a). The mechanisms of biotransformation of TNT by plants have been investigated in radiolabel mass balance studies, and shown to be considerable; degradation proceeds via reduction of the nitro-groups. A suite of known (2ADNT, 4ADNT, 24DANT; abbreviations given in Appendix D) and unknown (polar and non-polar) TNT metabolites was identified recently in poplar trees (Thompson and Schnoor 1996). Mineralization to CO_2 and formation of volatile organic compounds has been negligible (Palazzo and Legett 1986; Cataldo et al. 1989; Cataldo, Harvey, and Fellows 1990; Fellows, Harvey, and Cataldo 1995; Mueller et al. 1995; Thompson and Schnoor, 1996; Hughes et al. 1997; Price et al. 1997). Biotransformation of RDX in the presence of plants has been far lower than that of TNT, and accumulation of the parent compound depended upon plant species. Whether RDX degradation was carried out by the plants themselves or by microorganisms associated with plants was not verified; so far one RDX-degradation product has been identified in plant tissue (Cataldo, Harvey, and Fellows 1990; Fellows, Harvey, and Cataldo 1995; Price et al. 1997).

Phytoremediation of Explosives-Contaminated Groundwater from the Milan Army Ammunition Plant

The Milan Army Ammunition Plant (MAAP) located near Milan, TN (longitude 88° 50' W, latitude 35° 45'N) was selected as a demonstration site for phytoremediation of explosives on the basis of high concentrations of TNT and RDX occurring in groundwater (1.4 and 1.9 mg L^{-1}, respectively; TVA 1996, unpublished).

Phase I of this project, encompassing short-term plant screens for ability to remove TNT and RDX from groundwater, generated information on the basis of which suitable plant species were selected for Phase II, a field-scale wetland demonstration at MAAP.

The present study was undertaken to determine the behavior and fate of ^{14}C-labeled TNT and RDX in aquatic and wetland plants collected from the field-scale wetland demonstration with the objective of removing explosives from groundwater. The submersed species evaluated under hydroponic conditions were: *Elodea canadensis* Rich. in Michx. (elodea), *Potamogeton pectinatus* L. (sago pondweed), *Heteranthera dubia* (Jacq) Macm. (water star-grass); and the emergent species: *Myriophyllum aquaticum* (Vell.) Verdc., *Acorus calamus* L. (sweet-flag), *Phalaris aundinacea* L. (reed canary grass), and *Scirpus cyperinus* (L.) Kunth (wool-grass). The fate of TNT and RDX in the substrates in which the plants were rooted in the Milan Demonstration project was also assessed. Accumulation in plants and substrates over time, and biotransformation in plant portions and substrates, were evaluated.

2- Material and Methods

Hydroponic batch incubations of plant or substrate treatments with [^{14}C]-TNT or [^{14}C]-RDX were used to evaluate explosives transformation. A preliminary study exposing elodea to these respective compounds in separate 8- and 13-day incubations was carried out to evaluate experimental conditions and analytical techniques. The main study surveyed all seven species and two substrates in sequential, independent incubations of 7 and 13 days with TNT and RDX, respectively. Parent compounds and degradation products were determined through both radioisotope and chemical analyses of plant tissues, aqueous phases and substrate extracts.

[^{14}C]-TNT and [^{14}C]-RDX Specific Activity and Radiolabel Purity

The uniformly [^{14}C]-ring-labeled TNT (specific activity of 24.3×10^{7} Bq mmol^{-1}) and RDX (177.6×10^{7} Bq mmol^{-1}; NEN Research Products, Boston, MA) had 99% radiochemical purity as reported by the vendor. These purities were verified by diluting small aliquots in methanol, and analyzing by High Performance Liquid Chromatography /radiomatic (HPLC: Waters, see Analyses section; radiomatic: Series A-100 slow-1 beta unit, Packard Instruments, Downers Grove, IL; detection limit 6,500 disintegrations per minute (DPM), 92% efficiency). The purities of TNT and RDX were calculated by comparing DPM to total counts brought onto the HPLC column. The calculated purities matched the specifications provided by the vendor.

Plant Material, Substrates and Groundwater

The three submersed and four emergent plants evaluated for ability to take up and/or degrade explosives in MAAP groundwater were harvested in September 1996, from vegetation cultivated since April 1996 in explosives-contaminated groundwater in a purification lagoon and gravel beds at the Milan field site (Table 1). Whole plants were transported to WES in MAAP groundwater at ambient temperature and incubated the next day.

The submersed plants and the root systems of the emergent plants were freed from sediment by rinsing repeatedly with tap water. Submersed plants and the roots of the emergent plants were then immersed in 500 mL 1% H_2O_2 to reduce the presence of microorganisms. Plants were then rinsed in 500 mL de-ionized water, and adhering water was removed using paper towels.

The plant material used in the incubations represented whole plants as intact shoots or crowns. Each plant incubation consisted of three replicate beakers with each beaker containing 800-mL untreated groundwater and individuals of a species. For elodea and pondweed, approx. 10 g fresh weight (FW) was added to each beaker. For the other plant species, three representative plants were placed in each beaker. All fresh plant weights were determined before and after incubation, for both above-ground and below-ground portions.

Sediment and gravel were also collected from the Milan lagoon and gravel bed cells and incubated with groundwater. Each replicate consisted of 50 mL of substrate, approximately 26 g wet sediment or 20 g wet gravel, incubated with or without previous autoclaving (30 min at 50 psi). The chemical composition of the sediment and gravel were not determined; the composition of the sediment was expected to be similar to that of the wetted soil evaluated for explosives removal in earlier screening tests (Best and Sprecher 1996; Table 2).

Approximately 190 L of groundwater for incubation was collected from well M-146 at the end of August 1996. The water was pumped into a stainless steel drum, transported to WES overnight, and held at 10 °C. The groundwater was filter-sterilized over a 0.2 µm nylon filter to remove most microorganisms, collected in autoclaved 500-mL beakers and held in 1-L autoclaved, opaque Wheaton bottles in the refrigerator to minimize microbial contamination until use. Filtration decreased the concentration of NO_3-N considerably (from 6.0 to 0.1 mg L^{-1}) and the concentrations of TNT and RDX by 27% (Table 3, compare Appendix A). In comparison, explosives levels in the groundwater used in Phase I to screen species for explosives removal ability were 2197 µg L^{-1} and 3002 µg L^{-1} for TNT and RDX (TVA 1995; Best and Sprecher 1996). However, levels in well M-146 may have already been declining by then already.

Incubation Systems

To assess the potential for TNT and RDX transformation in the presence of plant tissue or substrate, and the related release of gaseous products into the atmosphere (volatilization/ aerosolization), the incubations were carried out in air-tight incubators with controlled air flow and individual gas-trapping systems. Two-piece, cylindrical incubators were constructed of 6 mm thick Plexi-glas. Those for submersed species, substrates and water controls were 41 cm high in total height x 30 cm diameter, and those for emergent species were 79 x 35.5 cm (Figure 1). Each incubator was equipped with an air inlet, and an outlet connected to a vacuum pump. The inlet was fitted with a one-way check valve to prevent air out-flow in the event of a power failure. Each incubator held one treatment, containing three replicate beakers of either groundwater with intact plants, groundwater alone, or groundwater with substrate. The capacities of the incubation beakers were 1.2 L for submersed plants, 2 L for emergent plants, and 1 L for controls (Table 4). The incubators were situated in a walk-in growth chamber. Illumination was at 500 to 600 μE m^{-2} s^{-1} for a 14-h photoperiod at a temperature of 23 °C. The air was pulled into the incubators by vacuum at 15 mm Hg, was passed successively through a 15 mL-XAD resin cartridge (to adsorb volatile organics), a gas-washing bottle containing 1 L 5N KOH (to trap CO_2), a condensation bottle (to trap KOH and moisture), and the pump itself. The pH of the KOH traps was maintained above 7 (unsaturated) by changing the chemical during the course of incubation (once a week).

Incubations

The filtered, explosives-contaminated MAAP groundwater used for incubations contained 988 μg TNT L^{-1} and 1,443 μg RDX L^{-1} (Table 3). Each replicate beaker contained 800 mL of this groundwater and was kept stirred magnetically. Each incubator holding three beakers rested on three stir-plates, one for each beaker. To prevent discontinuous rotation of the stirrer, magnetic bars were covered by stainless-steel gauze domes in the plant treatments, and substrates were suspended in stainless-steel baskets lined with aluminum foil. The groundwater was adjusted from pH 6.6 to 8 to prevent excessive CO_2 evolution into the atmosphere, and was amended with $NaHCO_3$ to an initial concentration of 298 mM to provide a carbon source for photosynthesis by the submersed plants. This was based on preliminary experiments, where elodea initially deteriorated rapidly but resumed growth after pH adjustment and bicarbonate amendment. Bicarbonate amendment was repeated once for the [^{14}C]-TNT incubations (after 3 days), and twice for the [^{14}C]-RDX incubations (after 3 and 7 days).

Radiolabel was initially added to each beaker as 1 mL methanol solution containing 55.5 x 10^4 Bq TNT or RDX as 519 or 69 μg explosive, respectively. Specific activity of TNT was 24.3 x 10^7 Bq $mmol^{-1}$ and of RDX 177.6 x 10^7 Bq $mmol^{-1}$. In the TNT incubations, radiolabel was re-dosed at mid-week, concomitant with addition of 2,175 μg unlabeled TNT per replicate. Total radiolabel used per replicate was 111.0 x 10^4 Bq TNT, or 55.5 x 10^4 Bq RDX (Table 5).

In the [^{14}C]-TNT exposures plants were incubated in solutions initially containing a total of 1,637 μg TNT L^{-1} (1,309 μg per 800mL replicate) with a total of 55.5 x 10^4 Bq radioactivity as 519 μg TNT per replicate (Table 5). The availability of unlabeled and labeled TNT was increased after

3 days of incubation by re-dosing to 3,367 µg L^{-1} (2,694 µg/800 mL replicate), and an additional 55.5 x 10^4 Bq per replicate. Actual total TNT concentration in the incubation water was initially slightly higher than at the Milan field site, i.e.1,637 µg L^{-1} (Table 5) versus 1,359 µg L^{-1} (Appendix A), respectively. Re-dosing with unlabeled analyte TNT was done to restore TNT levels presumed to be below detection after 3-day incubation (Best and Sprecher 1996; Best et al. 1997b) to a level similar to the TNT concentration of 2,200 µg L^{-1} previously measured in unfiltered MAAP groundwater. The TNT level of 3,367 µg L^{-1} in the incubation water after re-dosing was higher than initially.

In the incubations with [^{14}C]-RDX, plants were incubated in solutions initially containing a total of 1,529 µg RDX L^{-1} (1,223 µg/800mL) with a total of 55.5 x 10^4 Bq radioactivity as 69 µg RDX per replicate. This compared to 3,002 µg RDX L^{-1} found in unfiltered MAAP groundwater previously (Best and Sprecher 1996), and to 1,980 µg L^{-1} measured 11 September 1996 (Appendix A). Explosives levels in the groundwater from MAAP decreased between September 1995 and 1996.

The plants and substrates were placed into beakers filled with these groundwater/radiolabel solutions. For the submersed plants and substrates the beakers and their contents were fully exposed to illumination. For the emergent plants, sides and tops of beakers were covered with aluminum foil to prevent illumination of the roots. Two solution blanks without plants were also incubated with radiolabel: one illuminated, and one with side and top covered with aluminum foil to minimize photolysis.

Total radioactivity was determined in groundwater from each beaker using direct liquid scintillation (LS) counting of 3 1-mL samples per beaker at each of 3 sampling times, at the beginning (before addition of plants or substrates), at radiolabel re-dosing, and at the end of the incubation period. Radioactivity was also determined in acidified water samples at the end of the incubation period. Explosives contents of the incubated groundwater was determined at the end of the incubation period by HPLC, on one 100-mL sample per beaker (USEPA 1992).

Following incubation periods of 7 days for TNT and 13 days for RDX, the submersed plants and the root systems of the emergent plants were rinsed by blotting with paper towels, submersion in 500-mL de-ionized water, and additional blotting. Total fresh weight was determined per replicate. The emergent plants were then separated into above-ground and below-ground portions, and fresh weight was determined for these portions separately. One plant per treatment (i.e., per three replicates) was used for direct assessment of radiolabel distribution in the intact plant by radio-analytic imaging.

Plants of each replicate were clipped into small pieces (approx. 1 cm^3) using scissors, thoroughly mixed, wrapped in aluminum foil and stored deep-frozen (- 20 °C) until further tissue analysis. Following freezing, plants were ground with liquid N_2. Portions of 0.1 to 0.2 g FW per ground sample were weighed, and analyzed for radioactivity by combustion and subsequent LS. From submersed plants three sub-samples were analyzed per replicate; from emergents, three sub-samples from each above- and below-ground plant portion were analyzed.

The metal baskets containing the substrate treatments were removed from solution, and the standing water in each was decanted and returned to the incubation water in the beaker. The exterior of the substrates and baskets was freed from adhering incubation solution as described earlier in this section for plants. The substrates were transferred into pre-weighed glass jars, and total wet weight was determined for the substrate contents of each basket. The substrate samples were thoroughly mixed in the glass jars by stirring using a stainless steel scoop, and frozen until analysis. Portions of 0.14 to 0.90 g wet weight per mixed sediment sample, and 0.13 to 1.0 g wet weight (three small stones) per mixed gravel sample, were weighed, and analyzed for radioactivity by LS after combustion, i.e. three sub-samples per replicate.

Any material that may have condensed on the inside of the chamber following evapotranspiration was collected by wiping with paper tissue. The paper was weighed, clipped into small pieces, and mixed, and three sub-samples per incubator were analyzed for radioactivity by oxidation and LS.

Evolved [^{14}C]-CO_2 was quantified by LS of three sub-samples per incubator of the KOH solution in the gas-washing bottles. Resulting values were multiplied by the KOH volumes at the end of the incubation period. To account for changing the KOH solutions once a week, both KOH solutions per treatment were pooled before sub-sampling.

The volatilized [^{14}C]-organics were quantified by eluting the XAD-traps with methanol (4x void volume, 60 mL), LS of the solution, and multiplying the resulting value by the elution volumes (three sub-samples per incubator).

Analyses

Overview

LS determinations of total radioactivity in incubation solutions quantified all aqueous [^{14}C]-labeled compounds: explosives and their degradation products, as well as aqueous bicarbonate and CO_2 that became dissolved in the incubation water. The contribution of $^{14}CO_2$ and $H^{14}CO_3^-$ to the total radioactivity of the water was quantified by taking the difference between direct samples of the incubation water and those counted following acidification and aeration which removed aqueous CO_2 and bicarbonate.

LS determinations of total radioactivity in plants and substrates quantified all ^{14}C-labeled compounds. These could include explosives, known or unknown explosive degradation products, any photosynthetic metabolites resulting from plant assimilation of aqueous ^{14}C-bicarbonate and $^{14}CO_2$ generated during the incubation period, and ^{14}C produced from any plant- or substrate-associated microbial transformation. Direct LS of the filtered acetonitrile extracts of plants or substrates quantified the free, i.e. extractable, labeled compounds. In the preliminary experiments with elodea, acetonitrile extracts accounted for 27% of the total radiolabel in [^{14}C]-TNT exposed plants, and for 14% in [^{14}C]-RDX exposed plants. Radio-analysis by thin layer chromatography (TLC) of acetonitrile extracts was used to quantify groups of free radiolabeled compounds having similar mobilities. Identification was provided by comparison of Rf values of known, labeled, compounds. Unlabeled compounds, detectable by fluorescence under UV light, provided identification in certain cases. Chemical analysis by HPLC of acetonitrile extracts, following cleanup using Florisil cartridges, was then used to quantify that part of the free explosives and degradation products in plants and substrates that did not adsorb/adhere to the filters. In the elodea preliminary experiments, the radioactivities in these cleaned plant extracts were also determined by LS. Total radioactivities in these extracts accounted for 15% of the total radiolabel in the ^{14}C-TNT exposed plants, and for 5% in the ^{14}C-RDX exposed plants.

Radioactivity in Water and Other Liquids Using Liquid Scintillation Counting

The radioactivity in liquid samples was measured using a Liquid Scintillation Analyzer (Packard Instruments, Downers Grove, IL) with appropriate quench correction. Suitable aliquots of water (1 mL), KOH solution (0.5 mL), methanol (2 mL), or acetonitrile (100 µL), were placed in 20 mL glass vials with 15 mL scintillation cocktail (Ultima Gold, Packard Instruments, Meriden, CT). All samples were counted twice, each time for two min; only the data of the first count were used for the calculations, while those of the second count were used for verification. The detection limit was 50 DPM. Regularly run liquid standards indicated a typical counting efficiency of 98%.

Radioactivity in Plants and Substrates Using Combustion and Liquid Scintillation Counting

Plant, substrate and paper tissue samples were combusted in a Sample Oxidizer (Model 307, Packard Instruments, Meriden, CT) to determine the total amount of radioactivity associated with each sample. A predetermined weight of ground plant or substrate sample (approx. 0.1 g) was combusted at 900 to 1150 °C for 45 s under a stream of oxygen. The $^{14}CO_2$ evolved was trapped into 10 mL Carbosorb collected in standard counting vials. After adding 10 mL Permafluor (both solutions from Packard Instruments, Downers Grove, IL), the samples were counted using LS. After every other sample, a blank was burned consisting of an empty cellulose combustion cup. After every tenth sample a standard consisting of an empty cellulose combustion cup spiked with several µL explosives stock solution was burned. Counting of the standards indicated recovery efficiencies ranging from 93 to 97%.

Explosives in Water Using High Performance Liquid Chromatography

Analysis by direct injection on HPLC quantified explosives and their degradation products in groundwater (EPA 846 Method 8330; USEPA 1992). Water samples were filtered over 0.5 µm Millex-SR filters (Millipore, Milford, MA) prior to analysis. Concentration of the water samples by solid phase extraction (SPE) and by salting-out with NaCl (Jenkins et al. 1995) was tried prior to the current incubations. However, SPE cartridges were apparently plugged by mucilagenous plant excretion products, and salting-out gave low ^{14}C recoveries in the preliminary experiments with elodea, i.e. 25% with [^{14}C]-TNT and 0.04% with [^{14}C]-RDX, compared to LS determined totals. Therefore, determination by direct injection was used for analysis in the main incubations. While concentration methods such as SPE and salting out can increase accuracy of detection limits when amounts to be analyzed are low, direct injection does not provide the same low limits of detection.

HPLC methods for analysis of water were carried out following standard operating procedures for use of standards and controls, and for Quality Assurance/Quality Control (WES 1996). Determined were: HMX, RDX, TNB, Tetryl, TNT, 4ADNT, 2ADNT, 26DNT, 24DNT, 26DANT, 24DANT and the 4,4'-azoxy-derivative of TNT (2,2', 6,6 tetranitro- 4,4-azoxytoluene). Detection limits for target compounds in groundwater, following direct injection onto HPLC, were 0.020 mg L^{-1} . Exceptions were 2,4 DANT, 2,6-DANT and the 4,4-azoxy-derivative of TNT, with detection limits of 0.200 mg L^{-1}, 0.100 mg L^{-1}, and 0.500 mg L^{-1}, respectively. The HPLC system consisted of a Waters 610 Fluid Unit pump capable of achieving 6,000 psi, a Waters 717 plus Auto-sampler including a 200 µL loop injector, a Waters 486 Tunable UV Absorbance detector monitored at 245 nm and Millenium 2.1 Chromatography software (Waters Chromatography Division, Milford, MA). A Supelco LC-18 reverse phase HPLC column (25 cm x 4.6 mm, 5 µm; Catalog #5-8298) was used as the primary column and a Supelco LC-CN reverse phase HPLC column (25 cm x 4.6 mm, 5 µm; Catalog # 5-8231) as a confirmation column. As pre-column, the

Novapak C-18 (Catalog # WAT015220) or Novapak CN (Catalog # WAT020800; Waters Chromatography Division, Milford, MA) was used. A Cera Column Heater 250 at 30 °C (Catalog # 282-0252; Cera Inc., Baldwin Park, CA) was used to ameliorate retention time shifts due to changes in room temperature.

Explosives in Plants and Substrates Using High Performance Liquid Chromatography

Previously frozen plant samples were quick-frozen in liquid N_2 and ground to a fine powder. Then, 1.37 to 3.87 g FW portions per plant, or 3.12 to 5.83 g wet weight per sediment, or 3.03 to 5.12 g wet weight per gravel replicate were extracted in 10 mL acetonitrile by an 18-hr sonication in a water-cooled sonic bath. Temperature during sonication did not exceed 30 °C. Samples were centrifuged at 2,000 g for 5 min, the extract supernatant was freed from particles by filtering through a 0.50 µm Teflon disposable cartridge, and divided into two portions. One portion was used for direct LS, and for radioactivity and explosives analysis using TLC, and the other portion for explosives analysis using HPLC. The substrates were extracted similarly, without grinding. Polar as well as apolar metabolites of TNT and RDX were expected to appear in incubated plants and substrates; therefore, acetonitrile was used as the extraction solvent as it removes a range of non-polar to polar substances from organic materials, including amino-derivatives of explosives. Freeze-drying of the plant samples was omitted, because ^{14}C recoveries proved low in the preliminary experiments with elodea, i.e. 20% of that in fresh plant material for [^{14}C]TNT and 2% for [^{14}C]RDX, as determined by LS (without Florisil cleanup procedure).

For HPLC, a 0.5-mL extract portion was placed on a cleanup column prepared by layering 0.5 g Florisil and 0.5 g of neutral alumina. The column was washed with 5 mL of acetonitrile, and the resulting extract was diluted 1:1 with de-ionized water and analyzed by HPLC (EPA method 8330). HPLC methods for analysis of substrate extracts were carried out following standard operating procedures for use of standards and controls, and for Quality Assurance/Quality Control (WES 1996). Detection limits for target compounds in plant tissue ranged from 0.041 to 0.324 µg g^{-1} in elodea, and from 0.580 to 13.557 µg g^{-1} in sweet-flag on fresh weight basis, expressed as method detection level (MDL) (Appendix C). Detection limits in soil ranged from 0.5 to 2.0 µg g FW^{-1}.

Radioactivity Distribution in Intact Plants Using Autoradiography

Immediately following incubation, representative whole plant samples of submersed species, and apical and below-ground portions of emergent species, were placed on 20 x 20 cm glass plates, covered with radio-transparent mylar film, and scanned for 40 min. The Ambis Radioanalytic Imaging System (Ambis Inc., San Diego, CA), with a No. 4000 detector (sensitivity 0.07 DPM mm^{-2}) was used to quantify the radioactivity associated with intact plants. Radiographic images and associated radioactivities were stored on computer disk, and conventional camera photographs were taken of the plates.

Radioactivity in Plant and Substrate Extracts Using Thin Layer Chromatography and Autoradiography

For TLC analysis, a 3 to 5 mL acetonitrile extract aliquot was concentrated to approx. 1 mL by evaporation at 30 °C under N_2. Aliquots (20 to 50 µL) of this concentrated extract were analyzed for radiolabeled compounds following migration in various TLC solvent systems, by developing and radio-analytic imaging the plates. TNT and TNT-degradation products were separated from extracts of ^{14}C-TNT exposed plants or substrates on polar and fluorescent Silica Gel 60F plates (EM Science, Gibbstown, NJ), developed by incubation in a toluene:methanol (99:1 v/v) mixture solvent system for 40 min. TNT and known TNT metabolites separated well on these plates, and they could be identified by Rf value and by both color and fluorescence. RDX and RDX degradation products were separated from extracts of ^{14}C-RDX exposed plants or substrates using the same TLC plates as for TNT, and, in addition, on apolar Whatman Reversed Phase LKC18F plates (Octadecylsylane bonded; Whatman, Clifton, NJ), developed by incubation in a water:methanol (50:50 v/v) mixture solvent system for 4 h. Although RDX proved more mobile on the apolar than on the polar plate, most RDX degradation products remained immobile.

The presence of each compound or co-migrating group of compounds in the resulting chromatogram was determined by visual inspection under a fluorescent lamp (254 nm.; mineralight Model UVG-54, San Gabriel, CA). The radioactivity of each labeled compound was quantified by radioanalytic imaging (40 min). Radiographic images and associated radioactivities were stored on computer disk until further data processing. The identity of the compounds was determined by comparison of their Rf values with those of standards of either labeled TNT and RDX, or unlabelled 2ADNT, 4ADNT, 24DANT, 26DANT, 24DNT, and 26DNT, run on the same plate. The standard mix contained all these standards. The distribution of the radioactivity over the separated compounds was calculated relative to the total radioactivity per lane (chromatogram area allotted to each sample). Radiolabel recovery on the TLC plates was at least

20% of the total quantity applied, counted by LS. An example of the information provided by this TLC method is given in Figure 2.

Alkalinity, Macro-nutrients and Ions in Water

The filtered groundwater was analyzed for explosives as described above, and for pH, alkalinity, Kjeldahl-nitrogen (N), nitrate/nitrite-N, total-phosphorus (P), ortho-P, sulfate, calcium and iron at the beginning of the incubation.

The pH meter was calibrated with known buffer solutions bracketing the pH of the samples (American Public Health Association, APHA 1992). Alkalinity was determined colorimetrically as $CaCO_3$ (Method 310.2, USEPA 1979). Sulfate was determined colorimetrically (Method 375.2, USEPA 1979).

Kjeldahl-N and total P were measured colorimetrically in samples digested with sulfuric acid, potassium sulfate, and mercuric sulfate using a Lachat Quikchem AE Automatic Flow Injection Ion Analyzer (QuikChem Methods No. 10-107-06-2-D and No. 13-115-01-1-B, 1992). Ammonia-N was analyzed colorimetrically via the salicilate method using the Lachat System (QuikChem Method No. 12-107-06-2-A) and Nitrate/Nitrite-N was reduced over a cadmium column to Nitrite-N and analyzed colorimetrically via the Lachat system (Quikchem Method No. 10-107-04-1-C). Phosphate-P was analyzed colorimetrically using the Lachat System ascorbic acid method (QuikChem Method No. 12-115-01-1-A).

The concentrations of Ca and Fe were determined after acidification with 1:1 hydrochloric acid to pH<2 using Inductively Coupled Argon Plasma emission spectrometry (ICP; USEPA 1990 and USEPA 1992; SW-846 Method 6010).

3- Results and Discussion

Hydroponic studies addressed three objectives of importance to the field demonstration at MAAP, (1) To establish the physiological capacity of plants to absorb and transport TNT or RDX from explosives-contaminated groundwater in the absence of substrates (sediment, gravel) and their sorbing activities; (2) To quantify the partitioning of TNT and RDX over plant parts; (3) To establish the short-term chemical fate of TNT and RDX in plant tissues of these species. Substrates in which these plants were rooted at the Milan field site were also incubated without plants to investigate adsorption, and to evaluate microbial/chemical transformation of TNT and RDX that may affect explosives availability to plants.

Behavior of TNT in Hydroponic Culture

Plant Growth and Labeling in [^{14}C]-TNT Groundwater

Most plants decreased in weight over the 7-day incubation period in the TNT-amended incubation. Relative growth rates were usually negative (Figure 3; Appendix B - Table 1). Only the emergent parrot-feather thrived. Poor growth was generally attributed to lateness in the growth season (September) and TNT concentration approaching a toxic range for some aquatic plants (above 2.5 mg L^{-1} after re-dosing, cf. Schott and Worthley 1974; Smock, Stoneburner, and Clark 1976; Best et al. 1997a; $\geq$ 5 mg L^{-1} lethal after >2-week exposure for some aquatic and terrestrial plants, cf. Best et al. 1997a; Thompson and Schnoor, 1997). Emergent plants except parrot-feather may also have suffered from nutrient limitation, since they normally have access to interstitial sediment nutrient concentrations higher than those in the groundwater in the current incubations. The evapotranspiration rates in incubations with emergent plants were significantly higher than those with substrates and controls, concentrating the solution further (Figure 4; data in Appendix B - Table 2). However, expressed on above-ground dry weight basis, evapotranspiration rates of emergent plants were highly variable and not significantly different from each other (Figure 4; Appendix B- Table 2).

Radio-analytic imaging (Figure 5; Table 6) showed that in the submersed plants, the physiologically active leaves and roots were highly labeled. These species are known for carbon and nutrient uptake by leaves (elodea), or by leaves and roots (pondweed). In water star-grass, however, a gradient in label intensity was evident ranging from highest in leaves to lowest in roots. This indicates label uptake by leaves in elodea, by roots and leaves in pondweed, and mostly by leaves in water star-grass. In emergent plants, radiolabel was highest in roots, detectable in lower shoots, below detection in upper shoots, and again detectable in apical tips. This indicates label uptake by roots, limited transport upwards, and concentration in the physiologically active shoot tips.

Fate of [^{14}C]-TNT Radioactivity and Analyte TNT in Groundwater

Radioactivity in the groundwater decreased by a factor of two in most submersed plant incubations, but ten-fold with the three emergent species sweet-flag, reed canary grass and wool-grass (Table 7). Decreases were less in incubations with substrates than with submersed species, and decreases were not observed in groundwater controls. Groundwater TNT concentrations as determined by HPLC were undetectable in plant incubations after 7 days, accounting for a total disappearance of 4.003 mg TNT per beaker. The TNT residues (mg TNT per replicate) in the substrate incubations remained significantly higher in the autoclaved treatments than in the un-autoclaved ones (Table 7; Appendix B -Table 3). The TNT levels in groundwater controls remained relatively high, decreasing by 22% in darkness and by 40% in the light (Table 7), showing a significant effect of photolysis. The average radioactivity removal rates (calculated by dividing the difference between initial and final radioactivities of the incubation water by the number of incubation days), derived from these changes in concentrations were highest for the incubations with the three emergent species mentioned. TNT-equivalent removal rates were calculated as follows: e.g. for elodea, the radioactivity removal rate (3.3×10^6 DPM repl.$^{-1}$ d^{-1}) was divided by the total radioactivity per replicate (57.5×10^6 DPM replicate^{-1}), and multiplied by the total amount of TNT per replicate (4.003 mg replicate^{-1}) to give 0.23 mg repl.$^{-1}$ d^{-1}. These values were similar in the incubations with submersed plants and sediment, relatively higher with emergent plants, and lowest with gravel.

Specific, mass-based, removal rates were derived from the above-mentioned changes in radioactivity over time (Table 8). Specific TNT-equivalent removal rate was calculated as follows: e.g. elodea, the radioactivity removal rate (4.985×10^5 DPM g total DW^{-1} d^{-1}, Table 8) was divided by the total dose of radioactivity per replicate (57.5×10^6 DPM replicate^{-1}, Table 7), and multiplied by the total amount of TNT per replicate (4.003 mg replicate^{-1}, Table 7). Specific TNT-equivalent removal rates with plants were highest in the incubations with water star-grass (0.513 mg TNT-equiv. g total DW^{-1} d^{-1}) and lowest with sweet-flag (0.025 mg TNT-equiv. g below-ground DW^{-1} d^{-1}). These removal values correspond with 0.05 mg TNT g FW^{-1} d^{-1} in water star-grass and 0.001 mg TNT g total FW^{-1} d^{-1} in wool-grass. Specific TNT-equivalent removal with substrates was generally lower than with plants, highest with un-autoclaved sediment, and lowest with autoclaved gravel (Table 8). Adsorption or sorption of TNT to soils has been found to be low (Pennington 1988; Wood and Tiller 1996).

The endpoint explosives composition of the incubated groundwater (Figure 6; Appendix B- Table 3) differed greatly from that of the initial filtered groundwater as determined by HPLC (Table 3). The aqueous phase TNT concentrations were far lower in the plant and un-autoclaved substrate treatments, than in treatments with autoclaved substrates and controls. Little initial 2ADNT (9.3 $\mu g\ L^{-1}$) disappeared from most treatments with plants, increased with water star-grass (HD), but increased up to forty-fold in controls and with substrates. 4ADNT increased in all incubations, but to a lesser extent with most plants and controls, and more with substrates. Of the di-amino TNT-derivatives, 24DANT rose from non-detect to over 1 mg L^{-1} with pondweed (PP) and elodea (EC); 26DANT increased slightly. Traces of the 4,4-azoxy-derivative of TNT were found only in the gravel incubations, at 0.526 mg L^{-1} in one of the non-autoclaved gravel replicates. RDX decreased below detection limits with elodea, pondweed, and reed canary grass and decreased significantly with wool-grass. However, it was seen to increase significantly above dosage concentration with parrot-feather, sweet-flag, and to increase in some of the controls. Residues of 24DNT and TNB were below detection.

[^{14}C]-TNT Radioactivity Distribution over Plants, Substrates and Air

Radiolabel mass balances showed that in incubations with submersed plants and parrot-feather, about half of the [^{14}C]-TNT derived label ended up in the groundwater, and 24 to 79% in the plants (Table 9). With emergent plants most label was recovered in the plants; with substrate most label was recovered in the groundwater. Mineralization to aerial CO_2 was minimal, but $\geq$ 0.09 % in elodea, sweet-flag and most substrate treatments. Label incorporation into aqueous HCO_3 and CO_2 was usually significantly higher than mineralization. Incorporation into volatile organic compounds was negligible (maximally 0.3×10^6 DPM, collecting volatiles of three replicates per XAD trap). Most overall recoveries were within 67 to 118 %. High recovery in water star-grass (130%) could be explained by the high variability in radiolabel distribution over the plant (Figure 5; Table 6; and individual combustion values- not shown). Low recovery in reed canary grass, 60%, may be due to the evolution of methane, which was not recovered in the XAD and KOH traps. Reed canary grass is known for its ability to decrease oxygen levels rapidly in its rhizosphere (TVA, personal communication 1995), favoring chemical and/or microbial transformation of CO_2 to methane.

Label distribution varied over different plant species and organs (Table 10; Figure 5). The tissues of submersed plants incorporated more label than the above-ground portions of the emergent plants (radioactivity per g FW). In emergent plants, label was concentrated in the below-ground plant portions. Substrates incorporated less label than submersed plants; sediments incorporated approximately 1.5 x more than gravel, with a small decreasing effect of heat-inactivation.

Only a small part of the [^{14}C]-TNT-derived radiolabel associated with plant tissues, ranging from 9 to 33 %, proved 'free', i.e. un-conjugated into plant compounds and extractable in acetonitrile (Table 10). A similar ratio was extractable from the un-autoclaved substrates; however, more label was extracted from the autoclaved substrates (176% in autoclaved gravel and 464% in autoclaved sediment; calculated as average of three replicates) than found by combustion. The latter phenomenon may be explained by adsorption of TNT to the substrates and, consequently, high counts after combustion. This was verified as follows. An aliquot of [^{14}C]-TNT labeled solution with known strength was mixed with a known amount of autoclaved sediment, and a sub-sample was combusted and counted by LS (triplicate). The total radioactivity recovered by combustion of sediment sub-samples exceeded the radioactivity administered by 45%. Another explanation may be non-homogeneity of the substrates from which sub-samples were taken for combustion and extraction, and this is borne out by variability seen in autoclaved sediment (464 ± 428 %). Also, relatively more adsorption or transformation may have occurred at the substrates surface exposed to the labeled groundwater than at the unexposed substrate portions.

Fate of [^{14}C]-TNT Radioactivity and Analyte TNT in Plants and Substrates

The results of TLC analyses showed that most of the [^{14}C]-TNT-derived radiolabel in the plant and substrate extracts was polar, and did not move with the toluene:methanol solvent on the polar Silica Gel plates (Figure 7; Table 11). Labeled TNT was absent from all plants, except for the below-ground portion of reed canary grass, and from sediment. Labeled TNT was recovered in the autoclaved sediment, and in both un-autoclaved and autoclaved gravel, where it amounted to 9 to 17% of the radioactivity (Table 11). Radiolabel incorporation into ADNTs was found (2ADNT in submersed plants and substrates, and 4ADNT in emergent plants), but not into other known TNT degradation products. A total of five unknown labeled metabolites or groups of metabolites was found; three found only in plants (U3, U4, U5) and one found only in substrates (U2). The first unknown metabolite, U1, could be a mixture of more than one compound; its location at the origin suggests that it consists of polar compounds that did not migrate. These metabolites were not chemically identified. However, their behavior during separation by TLC could be characterized by mobility relative to standards and Rf. Relative mobilities were: DANTs<U2<ADNTs, and U3, U4 and U5>TNT on Silica Gel plates.

Explosives residues as determined by HPLC in plant tissue were limited to 4ADNT (and RDX; Figure 8; Appendix B- Table 4). 4ADNT concentrations were relatively high in the submersed plants (0.8 to 2.6 μg g FW^{-1}) and in the below-ground portions of parrot-feather (2.5 μg g^{-1}), reed canary grass (0.8 μg g^{-1}) and wool-grass (1.0 μg g^{-1}). RDX was only detected in water star-grass (1.0 μg g^{-1}) and in the below-ground portions of parrot-feather (2.0 μg g^{-1}). The 4,4-azoxy-derivative of TNT occurred in water star-grass (0.2 μg g^{-1}), and the below-ground portions of sweet-flag (1.9 μg g^{-1}) and reed canary grass (0.2 μg g^{-1}; Appendix B- Table 4). These residues of TNT metabolites are extremely low, even if 85% of total radioactivity was lost during clean-up of plant extracts, as is suggested by loss found in preliminary tests of elodea (see Materials and Methods, Analyses - Overview). They are far lower than earlier data on plant tissue residues in terrestrial plants, which were derived from radioactivity data and given as TNT equivalents, and which overestimated TNT since TNT (but not radiolabel) rapidly degrades (Cataldo et al. 1989). The present TNT metabolite residue levels in the plants are somewhat higher than found in more recent studies on terrestrial plants, indicating 4ADNT residues below detection (Fellows, Harvey, and Cataldo 1995). Comparison of the TNT and TNT metabolite residue levels of the present study with those found by Hughes et al. 1997 is not meaningful, since in the latter case plants were incubated in darkness and at high (30 to 95 mg L^{-1}), lethal TNT levels, conditions which did not allow normal plant metabolism-derived biotransformation of explosives. Low 4ADNT and RDX levels were found in the substrates, 0.25 and 0.50 μg g^{-1}, respectively. Autoclaving appeared to increase the 4ADNT and RDX residues in sediment by a factor 1.5 to 2, but only those of 4ADNT in gravel. Although autoclaving minimizes microbial activity in the substrates, it also changes the substrate structure, presumably increasing the adsorption sites. However, the latter adsorbed explosives remained extractable. No azoxy-compounds were recovered from the substrates.

Behavior of RDX in Hydroponic Culture

Plant Growth and Labeling in [^{14}C]-RDX Groundwater

Submersed species increased in weight over the 13-day incubation period in the RDX amended incubation. Relative growth rates were positive (Figure 9; Appendix B - Table 5), and those for elodea and pondweed were within normal ranges for field conditions at the end of the growth season (Best and Dassen 1987; Van Wijk 1989). Emergent plants decreased in weight, except for reed canary grass, which showed a growth rate considered normal for grasses at the end of the growth season. Emergent plants may have suffered from nutrient limitation, as in the

TNT amended incubations. However, wool-grass probably also suffered from root desiccation, since only 60 mL solution was left at the end of the incubation period. The evapotranspiration rates in the incubations with submersed plants and in the darkened control were significantly lower than in the remaining incubations (Figure 10; Appendix B- Table 6). In emergents, the trend in relative growth rate was reflected by the evapotranspiration rates, which were highest for reed canary grass.

Partitioning of radiocarbon in intact plants was assessed from the radio-analytic images (Figure 11; Table 12). The submersed plants were in general uniformly labeled. However, newly formed shoots of elodea and pondweed obviously served as either uptake site or sink for RDX, since they were very highly labeled. In the emergent plants, radiolabel was detectable in roots and in lower shoots, usually below detection in upper shoots, and extremely high in apical shoots. This indicates label uptake by roots, transport upwards, and concentration in the physiologically active shoot tips.

Fate of [^{14}C]-RDX Radioactivity and Analyte RDX in Groundwater

The radioactivity in the groundwater decreased by approx. 30 % in most plant incubations, by 79% with pondweed and by 91% with wool-grass (Table 13). Decreases in substrate incubations were 27 to 31% with sediment, and 4 to 6% with gravel. No decrease occurred in groundwater controls. The RDX concentrations as determined by HPLC (Table 13; Figure 12) decreased from the initial 1.5 mg L^{-1} by 40 to 50% of these levels in most plant and sediment incubations. Exceptions were RDX concentrations with pondweed (decreased by 98%) and wool-grass (by 100%), and with gravel (remained unchanged; Table 13). The radioactivity removal rates derived from these changes in concentrations were similar for the incubations with most plants and sediments, significantly higher with pondweed, and extremely low with gravel and in controls (Table 13). The analyte RDX removal rates generally reflected the radioactivity removal rates, but were usually somewhat higher than the RDX-equivalent removal rates.

Specific mass-based removal rates were derived from the above-mentioned changes in radioactivity over time (Table 14), and from the changes in analyte-RDX. RDX-equivalent removal rates were calculated as for TNT-equivalents. The RDX-equivalent removal rates indicated activity only in the plant treatments. The incubation with elodea had the highest activity (0.042 mg RDX-equiv. g DW^{-1} d^{-1}) and those with sweet-flag and wool-grass the lowest (0.007 mg RDX-equiv. g below-ground DW^{-1} d^{-1}). These removal values correspond with 0.004 mg RDX g FW^{-1} d^{-1} for elodea and 0.0002 mg RDX g total FW^{-1} d^{-1} for wool-grass. Specific analyte RDX removal rates were usually somewhat higher than the RDX-equivalent removal rates.

The explosives composition of the groundwater following incubation differed greatly from the initial filtered groundwater (Figure 12; Appendix B- Table 7; Table 3). TNT was only recovered in the controls, and had decreased significantly more in the light than in darkness. 2ADNT was below detection in all plant treatments, but had increased in the substrate treatments and controls; it was higher in the treatments with gravel and both autoclaved substrates than with plants. 4ADNT was below detection in all plant treatments except parrot-feather, and had increased in all non-plant treatments; it was significantly higher in gravel and both autoclaved substrates. Only very low levels of 24DNT were recovered in controls. TNB was below detection in treatments with plants or sediments, had decreased less with gravel, and least in controls. RDX had decreased in all plant treatments, except parrot-feather, and in sediment, but increased in the remaining substrate treatments, with parrot-feather and in the illuminated control. RDX increases in parrot-feather and control treatments may be due to the high evapotranspiration rates, which were particularly variable for parrot-feather (Figure 10; Appendix B - Table 6).

[^{14}C]-RDX Radioactivity Distribution over Plants, Substrates and Air

Radiolabel mass balances showed that in elodea, water star-grass, emergent plants (except for wool-grass), and in substrate treatments, most [^{14}C]-RDX-derived label ended up in the incubated groundwater, but that in pondweed and wool-grass treatments most label was recovered in the plants (Table 15). Mineralization to aerial CO_2 was generally low (< 1%), but higher in the sediment (2.08 %), pondweed (2.76 %), sweet-flag (4.06 %), reed canary grass (5.05 %) and particularly wool-grass (10.17 %). The pondweed and sediment incubations showed not only considerable $^{14}CO_2$ evolution, but also high (4 to 8%) incorporation of radiolabel in the aqueous HCO_3/CO_2. Incorporation into volatile organic compounds was negligible. Overall recoveries ranged from 56 to 112 %. Low recovery in wool-grass may be associated with some RDX crystal formation on the outside of the roots due to high solution loss. The latter radiolabel was not recovered, because it was probably rinsed from the plants after incubation (no radio-assay of the rinsing water was done).

Label distribution varied over plant species and organs (Table 16). The tissues of submersed plants and emergent plants were generally labeled to a similar extent. Exceptions were pondweed and the above-ground portions of reed canary grass, which incorporated relatively high amounts of label. Considerable root-to-shoot transport of label occurred in the emergent plants, as could be concluded by dividing the radioactivities in the shoots by those in the whole plants. It ranged from 23% of total plant radioactivity in sweet-flag to 81% in parrot-feather. Substrates were labeled far less than plants, with sediments approximately twice as much as gravel with no heat-inactivation effect.

A sometimes considerable part of the radiolabel found in the plant tissues, up to 61 %, proved 'free', i.e. extractable in acetonitrile (Table 16). The extractable fraction in the sediment was higher when autoclaved than un-autoclaved, 59% versus 25%, and was around 60% in gravel, where autoclaving did not appear to have an influence.

Fate of [^{14}C]-RDX Radioactivity and Analyte RDX in Plants and Substrates

Most of the [^{14}C]-RDX radiolabel in the tissue extracts of elodea, pondweed, and parrot-feather was incorporated into polar compounds that did not move with the toluene:methanol solvent on the polar Silica Gel plates (Table 17; Figure 13). Labeled RDX was detected in the acetonitrile extracts of all plants and substrates using separation of compounds by TLC on Silica Gel plates. RDX accounted for $\leq$2 to 63% of the radioactivity in the plant extracts, and $\leq$20 to 80% of the radioactivity in the substrate extracts. Substantial amounts of labeled compounds comigrated with RDX in this TLC system (polar Silica Gel) in extracts of water star-grass and most of the emergents. However, since these activities were often higher than those separated using a TLC system with higher resolution for RDX (apolar Whatman plates; Table 18; Figure 14), it was concluded that in the Silica Gel separation the RDX was accompanied by (an) unknown metabolite(s). A total of five spots attributable to labeled RDX metabolites was found, of which one (polar, U4) was found only in two plant species. The spots U1 and U3 could represent mixtures of more than one compound. These metabolites were not chemically identified. Their mobilities relative to known compounds were: DANTs<U2<ADNTs and equal to RDX on Silica Gel plates; U4 and U5>RDX on Whatman plates.

Explosives residues as determined by HPLC in plant tissue were limited to RDX (and 4ADNT; Figure 15; Appendix B- Table 8). RDX was detected in all plants; however, levels were below detection in the below-ground portions of sweet-flag and wool-grass. RDX residues ranged from 0.32 μg g FW^{-1} in pondweed to 8.57 μg g FW^{-1} in parrot-feather shoots. These RDX concentrations are extremely low, even if 95% was lost during cleanup of the plant extract, as suggested by recoveries in preliminary elodea incubations (see Materials and Methods, Analyses - Overview). They are far lower than plant tissue residues derived from radioactivity data elsewhere and given as RDX equivalents; the latter may have overestimated RDX somewhat since RDX (but not radiolabel) degrades rather slowly (Cataldo, Harvey, and Fellows 1990). The present RDX residue levels in the plants are somewhat lower than more recent data on terrestrial plants (>18 μg g^{-1} in corn and >180 μg g^{-1} in alfalfa; Fellows, Harvey and Cataldo 1995), and similar to those recovered in recent similar mass balance studies of terrestrial plants (maximum of 16 μg g^{-1} in foliage; Price et al. 1997). No explosives nor known metabolites were recovered in the substrates. 4ADNT concentration was considerable in the below-ground portions of parrot-feather. Azoxy-compounds were absent from all plants and substrates evaluated.

General Discussion

Behavior of TNT and RDX in Groundwater

Although plants did not grow well, TNT was removed, and RDX was greatly decreased in treatments with plants. TNT disappearance from groundwater incubated with plants over 7 days was associated with the subsequent presence of explosive-derived radioactivity in plant tissues. Highest specific TNT removal rates were found in submersed plants in star-grass (0.05 mg TNT g FW^{-1} d^{-1}), and in emergent plants in parrot-feather, sweet-flag, and reed canary grass (0.006 mg TNT g total FW^{-1} d^{-1}). RDX disappeared less rapidly than TNT from the incubated groundwater, and was associated with the subsequent presence of explosives-derived radioactivity in plant tissues. Highest specific RDX removal rates were found in submersed plants in elodea (0.004 mg RDX g FW^{-1} d^{-1}), and in emergent plants in reed canary grass (0.001 mg RDX g total FW^{-1} d^{-1}).

The more rapid decrease in TNT levels in illuminated controls than in dark controls without plants supports photolysis of TNT (Spanggord et al. 1980; Gorontzy et al. 1994), similar to that reported for dissolved organic matter (DOM) originating from both live and decomposing plants in water bodies (Wetzel, Hatcher, and Bianchi 1995). The more rapid decrease in TNT and RDX levels with plants may also be due to the generation of plant-specific DOM and leachates, both providing small fatty acids or assimilates readily available to microbes (Wetzel, Hatcher, and Bianchi 1995; Mann and Wetzel 1996), and enhancing microbial degradation of explosives. These products may have given the problems in SPE columns.

Behavior of TNT and RDX in Plants

The behavior of TNT and RDX seen in this study does, in fact, generally follow that of a herbicide in contact with plants. Plant detoxification of herbicides (Kreuz, Tommasini, and Martinoia 1996; Trapp and Matthies 1995) is generally enzyme-mediated, in which a primary step often includes oxidation or hydrolysis, which may provide a functional group suitable for subsequent covalent binding to an endogenous moiety. This first step often results in the formation of glycosides. Another important conjugation reaction in plant herbicide metabolism is that with the major cellular thiol, GSH (γ-glutamyl cysteinyl glycine); this conjugation was shown to occur in plants tolerant to atrazine under aerobic conditions . It is noted that atrazine is a herbicide similar in structure to RDX. The resulting conjugates are 1) generally inactive toward the initial target site, 2) more hydrophilic and less mobile in the plant than the parent compound, and 3) susceptible to further processing which may include secondary conjugation, degradation, and compartmentalization. Metabolism of herbicides to glycosides or to GSH conjugates is usually considered a detoxification process, but the products are not always themselves benign and may possess toxicological activities. Recent unpublished work indicates incorporation of amino transformation products of TNT in coniferyl alcohol, a precursor of lignin, in tree species (K.Thorn, USGS, unpublished 1997). This finding supports the hypothesis that explosives-tolerant and -degrading plants may possess detoxification mechanisms similar to those identified in herbicide-resistant agricultural crops, and that the degradation products are utilized as secondary plant substances.

In the present study, specific, mass-based, TNT-equivalent removal rates, derived from the changes in [^{14}C]-TNT derived radioactivity over time, were far higher in the incubations with submersed plants than in those with emergents. Plant tissue labeling strength was consequently higher also. A relatively small part of the tissue radiolabel was 'free', i.e. non-conjugated, and extractable. Radiolabel mass balances indicated considerable [^{14}C]-TNT derived label incorporation into plants, low mineralization to CO_2/HCO_3, and negligible evolution into volatile organic compounds. The [^{14}C]-TNT-derived radiolabel was taken up by physiologically active roots and leaves in submersed plants, and appeared to remain at the sites of uptake. The label was taken up by the roots of emergent plants, and it was transported to a limited extent in an apical direction. TNT may have been transformed (conjugated) prior to transport (Cataldo et al. 1989; Michels and Gottschalk 1994; Fellows, Harvey, and Cataldo 1995; T.F.Jenkins 1996, unpublished; Thompson and Schnoor 1996), since virtually no labeled TNT residues were recovered in the plant extracts after 7 days. [^{14}C]-TNT was reduced to 2ADNT in submersed plants and to 4ADNT in emergent plants. Five other unknown metabolites or groups of metabolites were separated by TLC, but not identified. These metabolites can be attributed to plant activity. No evidence for degradation via nitro-group removal, i.e. no DNTs, was found. Only a small quantity of labeled TNT remained in the below-ground portions of one species, reed canary grass. HPLC analysis confirmed the presence of 4ADNT, but not of TNT, in most plant tissues. The absence of labeled 4ADNT in some plants after 7 days does not preclude its presence and subsequent transformation into other unknown metabolites. Toxic azoxy-compounds had accumulated only in water star-grass and in the below-ground portions of sweet-flag and reed canary grass. HPLC analysis proved RDX to be absent from most plants, except in water star-grass and the below-ground portions of parrot-feather.

Specific, mass-based, RDX-equivalent removal rates, derived from the changes in [^{14}C]-RDX derived radioactivity over time, were highest in the incubations with elodea. The tissues of submersed plants incorporated as much as those of most emergent plants. A relatively larger part of the tissue radiolabel than in the case of TNT was 'free'. Radiolabel mass balances indicated considerable label incorporation in plants, some mineralization to CO_2/HCO_3, and negligible evolution into volatile organic compounds. The [^{14}C]-RDX derived radiolabel was probably taken up over the whole plant surface of the submersed plants, but it tended to accumulate in newly-formed shoots. The label was taken up by the roots of emergent plants, and considerable transport in an apical direction took place. Part of the RDX was probably transformed (conjugated) prior to transport (this part was relatively higher than in the case of TNT) (Cataldo, Harvey, and Fellows 1990; Fellows, Harvey, and Cataldo 1995; T.F.Jenkins 1996, unpublished), since only part of the labeled RDX residues was recovered in the plant extracts after 13 days. The five unknown metabolites separated by TLC were not identified. One unknown, U4, was unique to plants, but the other four were also found in substrates. The lowest [^{14}C]-RDX level occurred in pondweed and was close to the detection limit for radio-analytic imaging. Analyte RDX concentrations determined by HPLC were usually higher in emergent than in submersed plants, and accumulation occurred in the above-ground plant portions. Azoxy compounds were not found.

To address the question of which plant species would be most effective in a constructed wetland with the objective of removing explosives from groundwater, several plant characteristics have to be taken into account. These are the high, specific, plant mass based explosives removal rates, which were 4 to 8 x higher for TNT, and 2 to 10 x higher for RDX in submersed than in emergent plants, and the high metabolization of the parent explosives, almost complete for TNT in all plants, highest for RDX in elodea and pondweed. From these results, submersed plants, particularly elodea and pondweed, would be most suitable. However, emergent plants can be as effective as submersed species per wetland unit area, presuming that removal rate and metabolic activity are proportional to standing crop (g plant mass produced per m^2), which is typically 2 to 5 x higher in emergent species.

Behavior of TNT and RDX in Substrates

Specific mass-based TNT-equivalent removal rates in the incubations with substrates were generally lower than with plants. Substrate labeling strength ranked between that of submersed plants and that of below-ground portions of emergent plants. Although [^{14}C]-TNT incorporation/adsorption in sediment and gravel was considerable, no analyte TNT was recovered in the substrate extracts by HPLC analysis. Labeled TNT was recovered in the extracts of three of the four substrates using TLC. Part of the TNT had been transformed, with metabolite(or group of metabolites) identical to that extracted from plants (ADNTs), and one unique for what was presumably the microbial component of the substrates (U2). HPLC analysis confirmed the presence of 4ADNT (and of RDX) in all substrates. No evidence for TNT degradation via nitro-group removal was found.

Specific mass-based RDX-equivalent removal rates in the substrate incubations were negligible. Specific analyte RDX removal rates were extremely low. [^{14}C]-RDX incorporation/ adsorption, as determined by combustion was very low in sediment and gravel. Labeled RDX was recovered in extracts of all substrates using TLC. RDX residues proved also detectable by HPLC analysis. A relatively small part of the [^{14}C]-RDX had been transformed, with four metabolites identical to those extracted from plants. However, HPLC analysis did not identify known RDX degradation products (MNX, TNX).

Summary

This mass balance shows that:

1. TNT was rapidly (0.001 mg TNT g total FW^{-1} d^{-1} in wool-grass to 0.05 mg TNT g FW^{-1} d^{-1} in water star-grass) transformed by explosives-adapted emergent and submersed plants. Neither periphyton nor substrates (sediment and gravel) significantly contributed to this transformation.
2. Mono-aminodinitrotoluene and di-amino-dinitrotoluene levels did not accumulate in the incubation water. Azoxy compounds were only recovered from water from incubations with gravel.
3. 2ADNT levels decreased in groundwater incubated with plants, but 2ADNT was not recovered from plant tissues; 4ADNT levels remained unchanged in groundwater with submersed plants but increased with emergent plants. 4ADNT appeared in the tissues of submersed plants and in the below-ground parts of emergent plants.
4. 24DANT and 26DANT levels increased in groundwater incubated with submersed plants, but neither compound was recovered from plant tissue.
5. Five TNT transformation products were separated from plant and substrate extracts using TLC, but not identified. Three products occurred only in plants. Azoxy compounds had accumulated only in one submersed and in the below-ground portions of two emergent plants.
6. Only a small amount of TNT-derived ^{14}C was extractable with solvent from plant tissue which became radiolabeled.
7. Mineralization of TNT, i.e. $^{14}CO_2$ evolution, was extremely low.
8. Photolysis of TNT was demonstrated in the illuminated water control.

9. RDX was rapidly (0.0002 mg RDX g total FW^{-1} d^{-1} in wool-grass to 0.004 mg RDX g FW^{-1} d^{-1} in elodea) transformed by explosives-adapted emergent and submersed plants. Transformation was slower than that of TNT. Neither periphyton nor substrates (sediment and gravel) significantly contributed to this transformation.
10. RDX levels remained detectable in the incubation water of all plant treatments, except wool-grass. No known RDX transformation products were found in the water.
11. RDX and five RDX transformation products were separated from plant and substrate extracts using TLC, but not identified. One product occurred only in plants.
12. Only a small amount of RDX-derived ^{14}C was extractable with solvent from plant tissue which became radiolabeled.
13. Mineralization of RDX, i.e. $^{14}CO_2$ evolution, was extremely low.

The promise of phytoremediation in constructed wetlands as a technology for removal of explosives from groundwater is supported by several results of this study. 1) The rapid decrease in TNT and relatively slower decrease in RDX in the presence of certain aquatic or wetland plants under viable environmental conditions, 2) The relatively rapid metabolism of the parent compounds inside the plants, and 3) Low explosives residues in plant tissues and substrates. However, it must be realized that metabolic pathways of degradation of TNT and RDX in plants are still unknown, that certain explosives degradation products may exert other biological and toxicological activities, and that decreases in TNT and RDX levels in water with plants may also partly be due to chemical binding between explosives transformation products and organic matter, or to the generation of plant-specific dissolved organic matter and leachates, both stimulating microbial activity and resulting in degradation of explosives.

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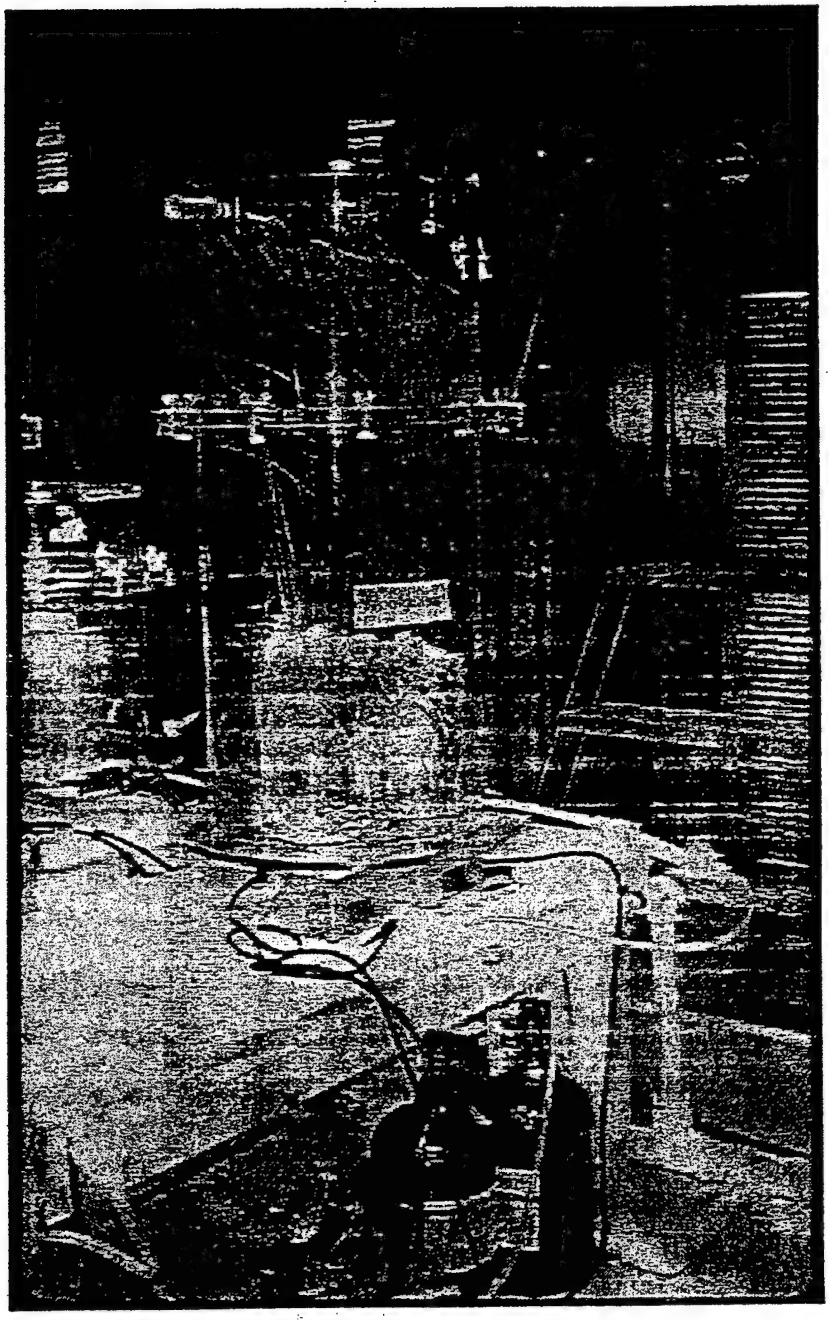
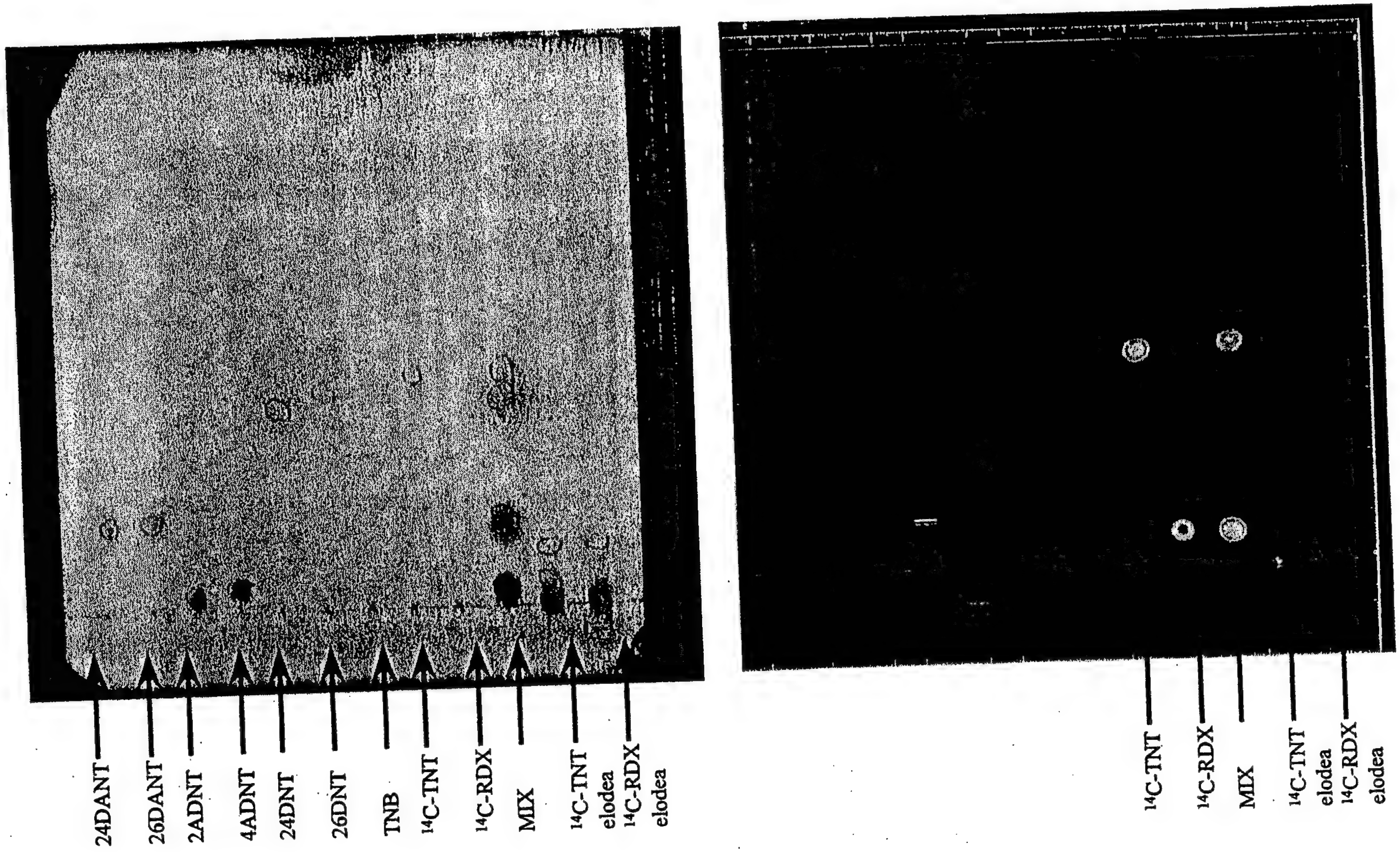


Figure 1. Incubation systems used for mass balance studies.


24DANT
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^{14}C-RDX
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^{14}C-RDX elodea
^{14}C-TNT
^{14}C-RDX
MIX
^{14}C-TNT elodea
^{14}C-RDX elodea

Figure 2. Conventional camera photograph with fluorescent illumination (left side) and radio-analytic image of a thin layer chromatogram (right side) used to separate radiolabeled and unlabeled explosives and degradation products.

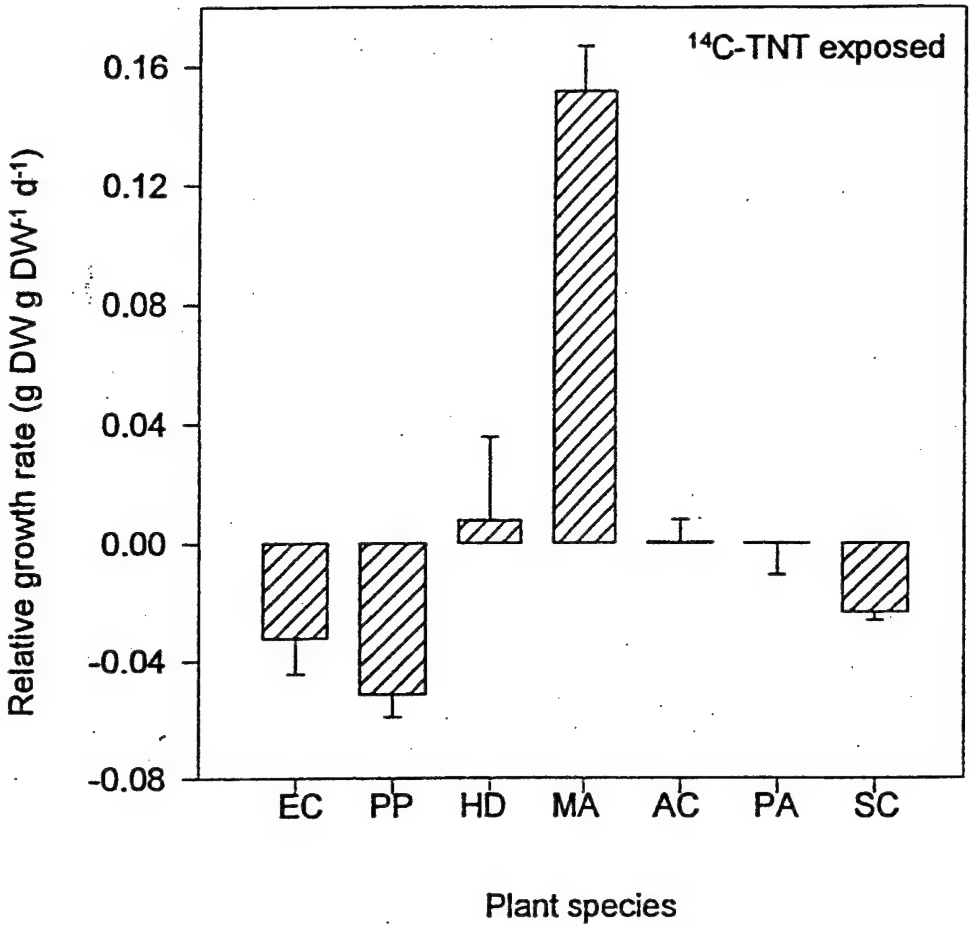

Figure 3. Relative growth rates of plants over 7-day incubation in [^{14}C]-TNT amended groundwater containing 1.64 to 3.37 mg TNT L^{-1}. Mean values and standard deviations(N=3). Abbreviations: EC, elodea; PP, pondweed; HD, water-stargrass; MA, parrot-feather; AC, sweet-flag; PA, reed canary grass; SC, wool-grass.

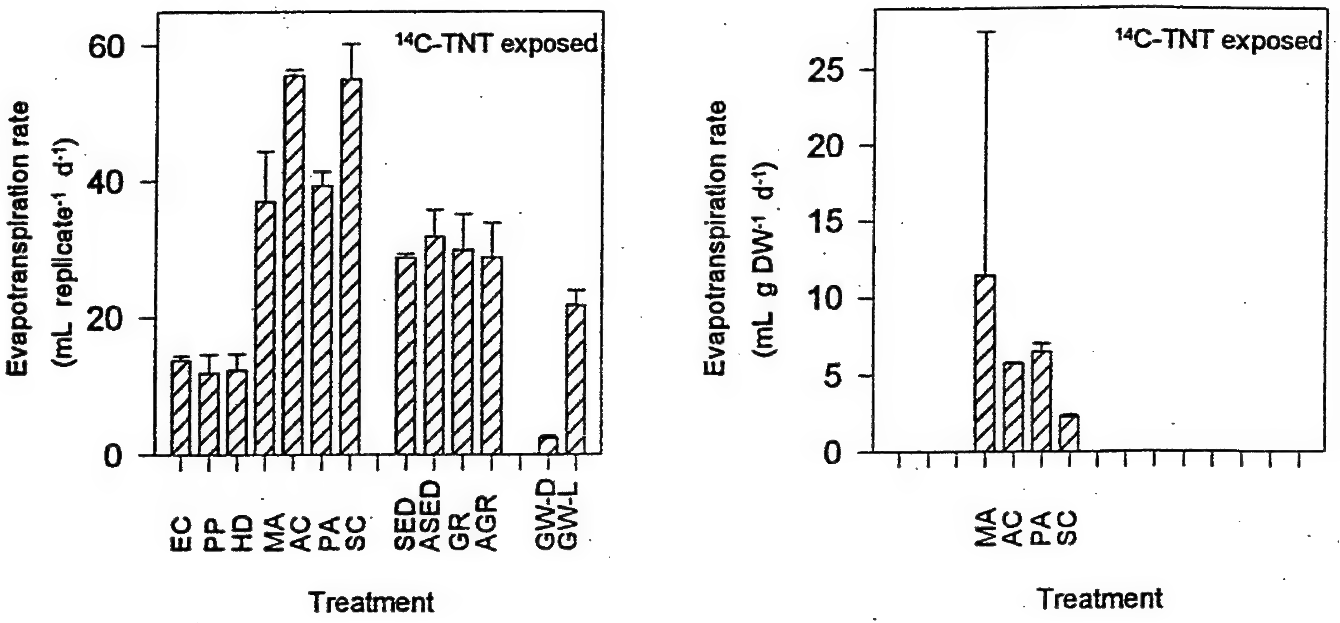

Figure 4. Evapotranspiration rates in [^{14}C]-TNT groundwater over 7-day incubation with plants, substrates, or controls. Mean values and standard deviations (N=3). Abbreviations: EC, elodea; PP, pondweed; HD, water-stargrass; MA, parrot-feather; AC, sweet-flag; PA, reed canary grass; SC, wool-grass; SED, sediment; ASED, autoclaved sediment; GR, gravel; AGR, autoclaved gravel; GW-D, groundwater darkened; GW-L, groundwater illuminated.

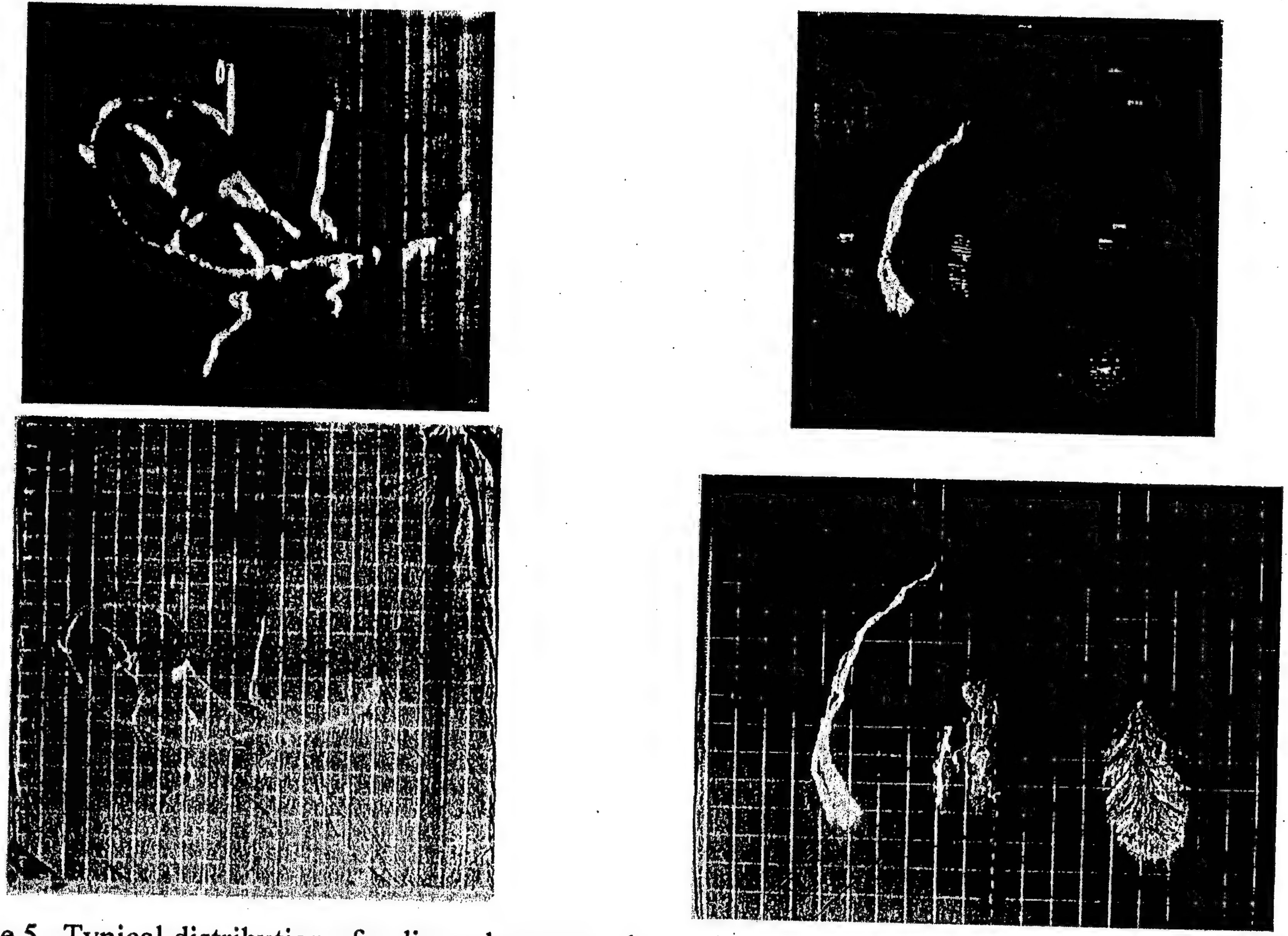

Figure 5. Typical distribution of radio carbon over plant organs or portions after 7-day incubation in [^{14}C]-TNT explosives-contaminated groundwater, as indicated by radio-analytic imaging (upper). Conventional camera-photographs provided visual information on plant morphology and orientation on the plant-supporting glass plate (lower).

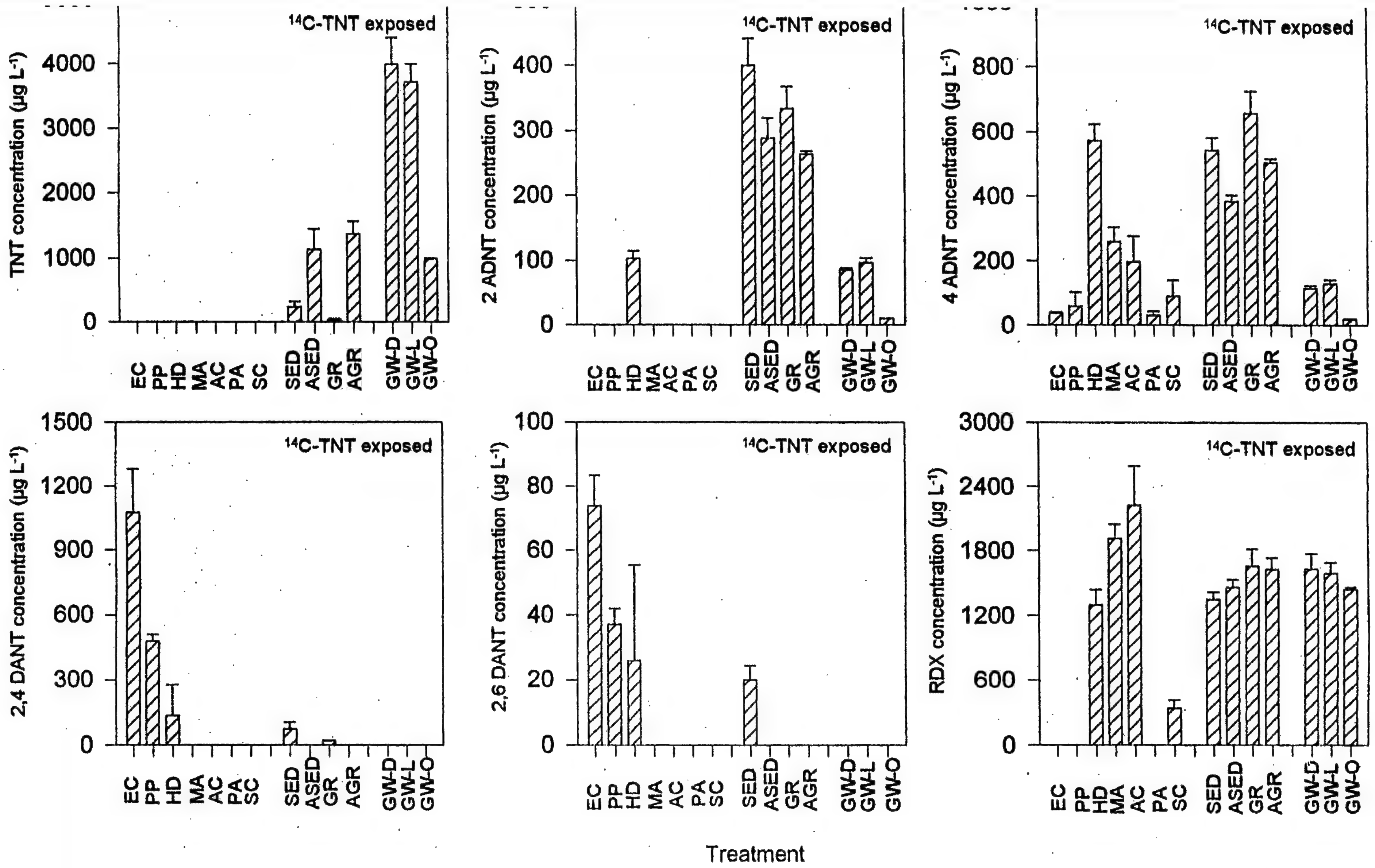

Figure 6. Explosives concentrations in [^{14}C]-TNT groundwater after 7-day incubation with plants, substrates, controls, and initially, as determined by HPLC analysis. Mean values and standard deviations (N=3). Abbreviations: EC, elodea; PP, pondweed; HD, water-stargrass; MA, parrot-feather; AC, sweet-flag; PA, reed canary grass; SC, wool-grass; SED, sediment; ASED, autoclaved sediment; GR, gravel; AGR, autoclaved gravel; GW-D, groundwater darkened; GW-L, groundwater illuminated; GW-O, initial groundwater.

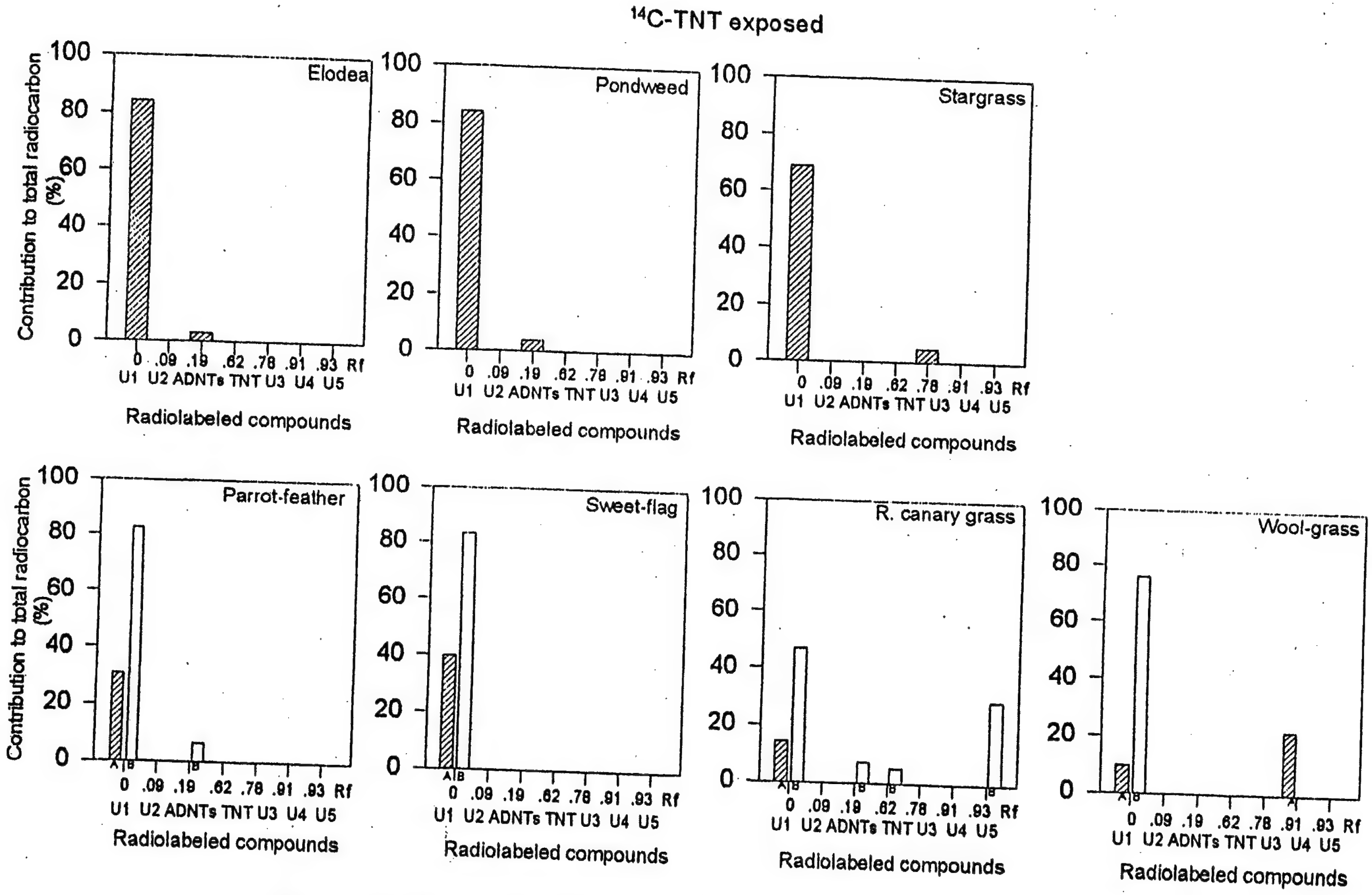

Figure 7. Distribution of TNT-derived radioactivity over acetonitrile extracted compounds from plants and substrates, expressed as percent of total counts per TLC lane. Separation by thin layer chromatography of extracts and references on Silica Gel 60F plates in a toluene:methanol mixture (98:2), using the Ambis Radioanalytic Imaging System for measuring radioactivity. Abbreviations: A, aboveground; B, below-ground; U, unknown.

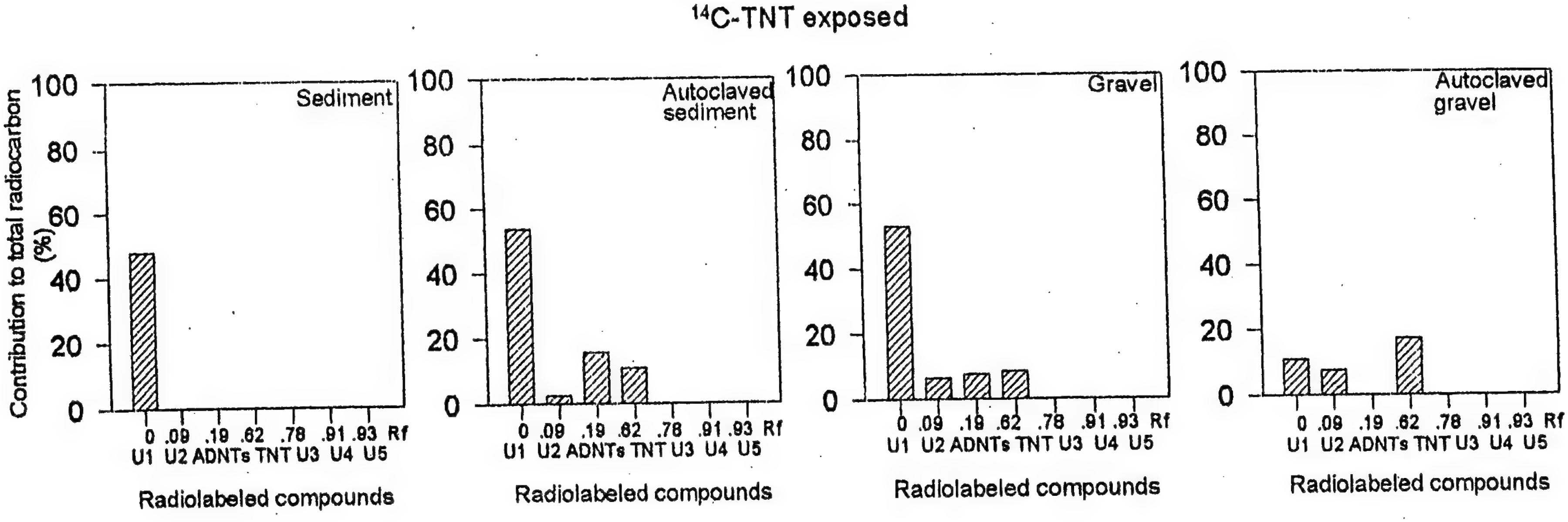

Figure 7- continued.

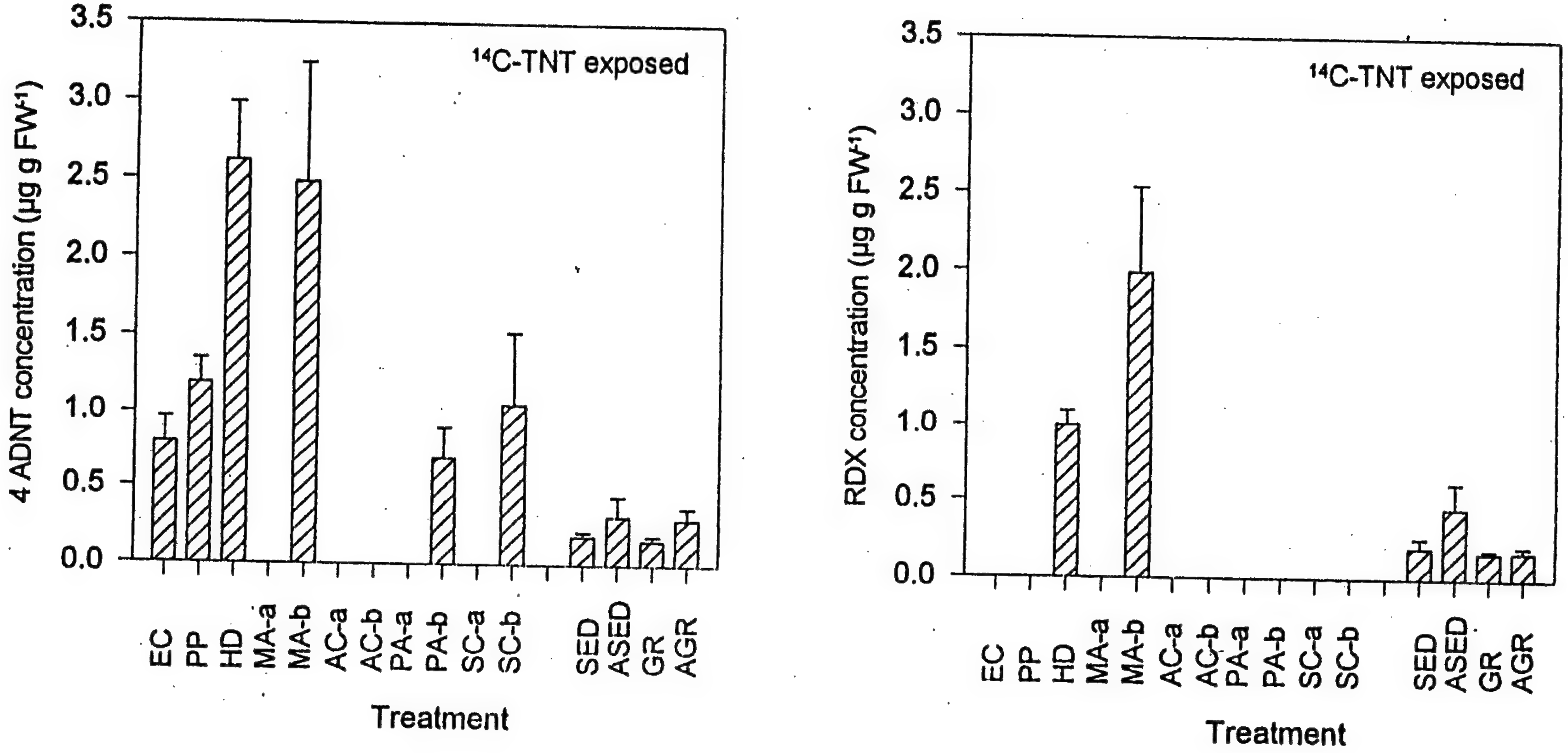

Figure 8. Explosives residues in plants and substrates after 7-day incubation in [^{14}C]-TNT groundwater, as determined by HPLC analysis. Mean values and standard deviations (N=3). Abbreviations: EC, elodea; PP, pondweed; HD, water-stargrass; MA, parrot-feather, -a above-ground and -b below-ground; AC, sweet-flag, -a above-ground and -b below-ground; PA, reed canary grass, -a above-ground and -b below-ground; SC, wool-grass, -a above-ground and -b below-ground; SED, sediment; ASED, autoclaved sediment; GR, gravel; AGR, autoclaved gravel.

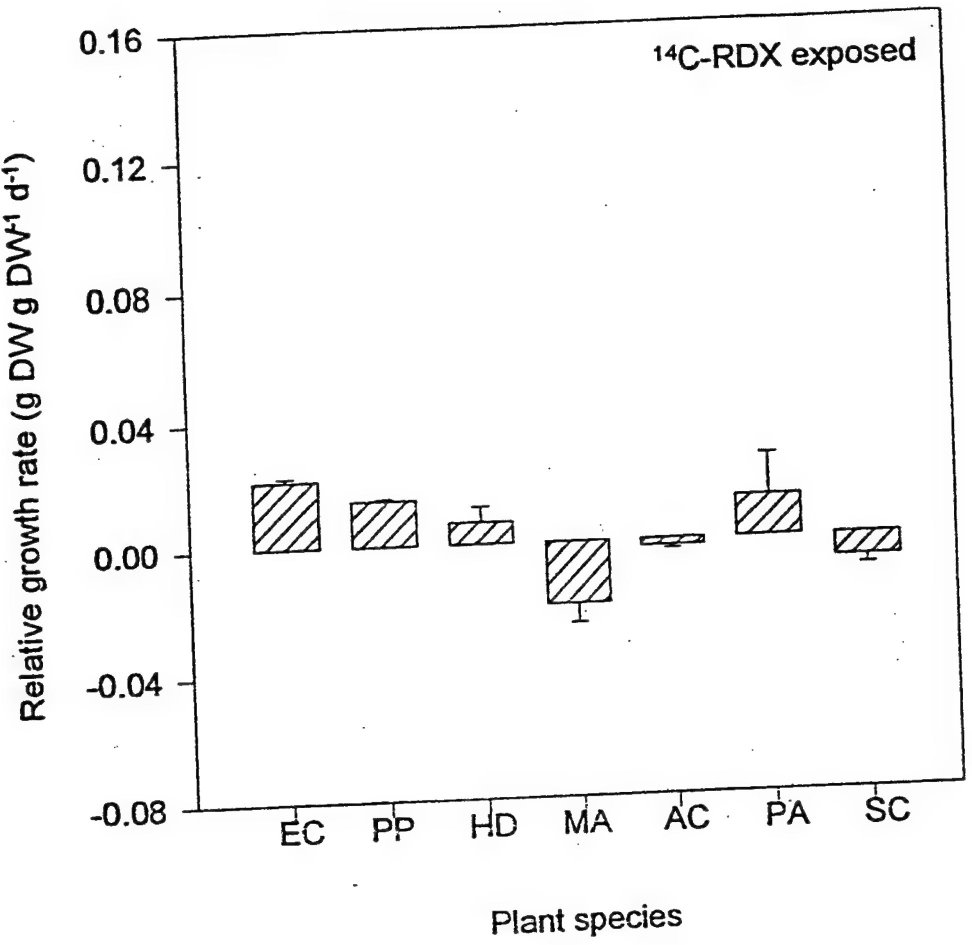

Figure 9. Relative growth rates of plants over 13-day incubation in [^{14}C]-RDX amended groundwater containing up to 1.53 mg RDX L^{-1}. Mean values and standard deviations (N=3). Abbreviations: EC, elodea; PP, pondweed; HD, water-stargrass; MA, parrot-feather; AC, sweet-flag; PA, reed canary grass; SC, wool-grass.

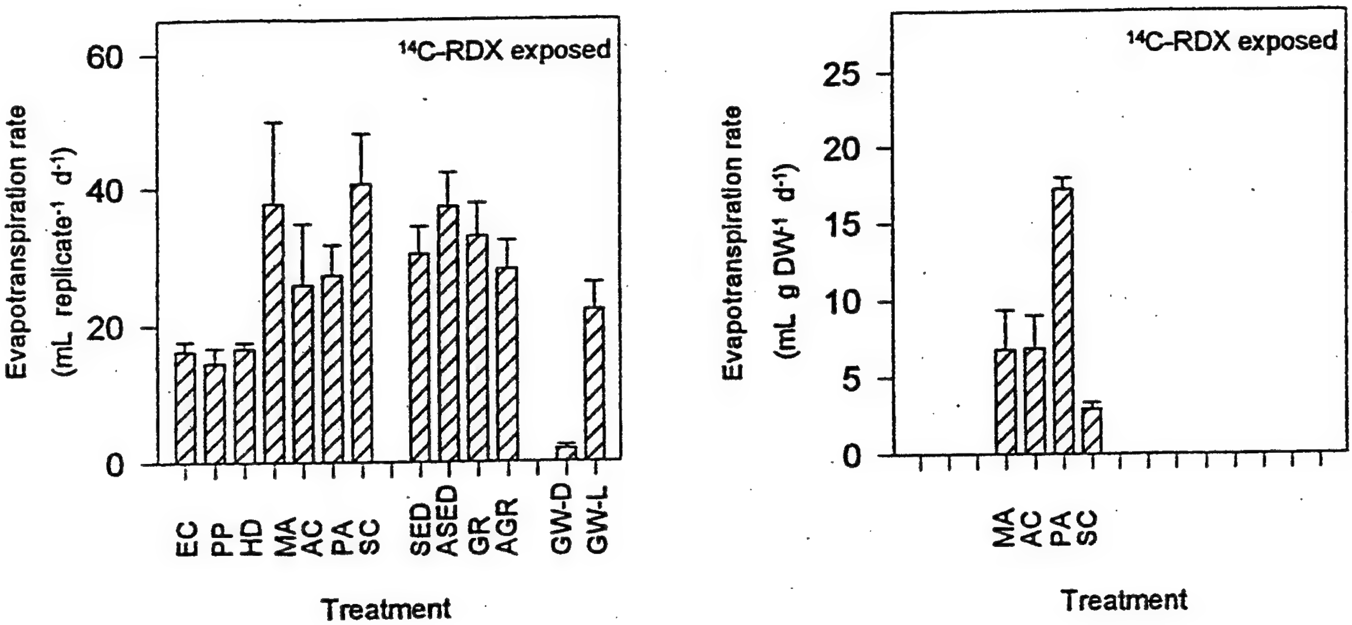

Figure 10. Evapotranspiration rates in [^{14}C]-RDX groundwater over 7-day incubation with plants, substrates, or controls. Mean values and standard deviations (N=3). Abbreviations: EC, elodea; PP, pondweed; HD, water-stargrass; MA, parrot-feather; AC, sweet-flag; PA, reed canary grass; SC, wool-grass; SED, sediment; ASED, autoclaved sediment; GR, gravel; AGR, autoclaved gravel. GW-D, groundwater darkened; GW-L, groundwater illuminated.

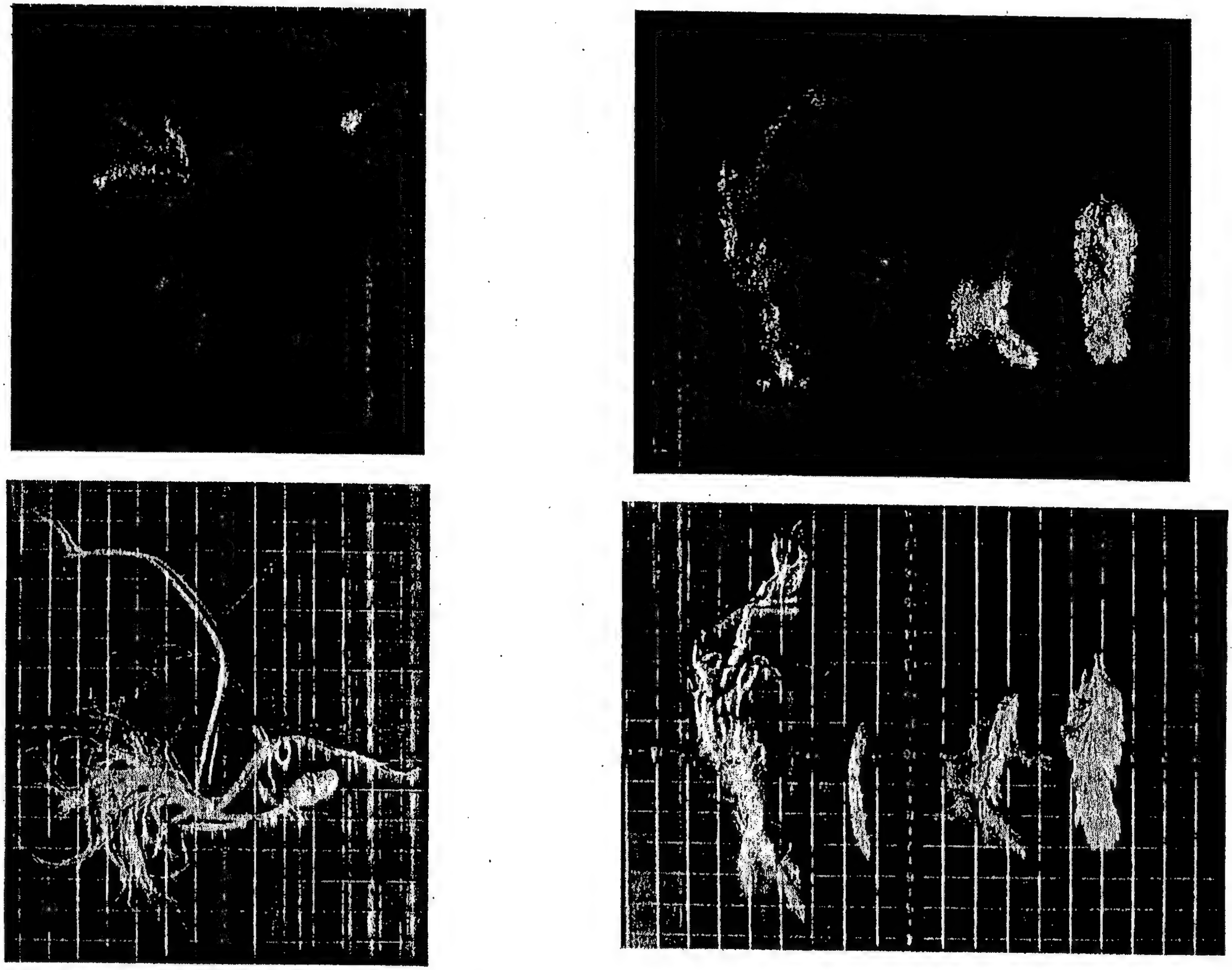

Figure 11. Typical distribution of radio carbon over plant organs or portions after 13-day incubation in [^{14}C]-RDX explosives-contaminated groundwater, as indicated by radio-analytic imaging (upper). Conventional camera-photographs provided visual information on plant morphology and orientation on the plant-supporting glass plate (lower).

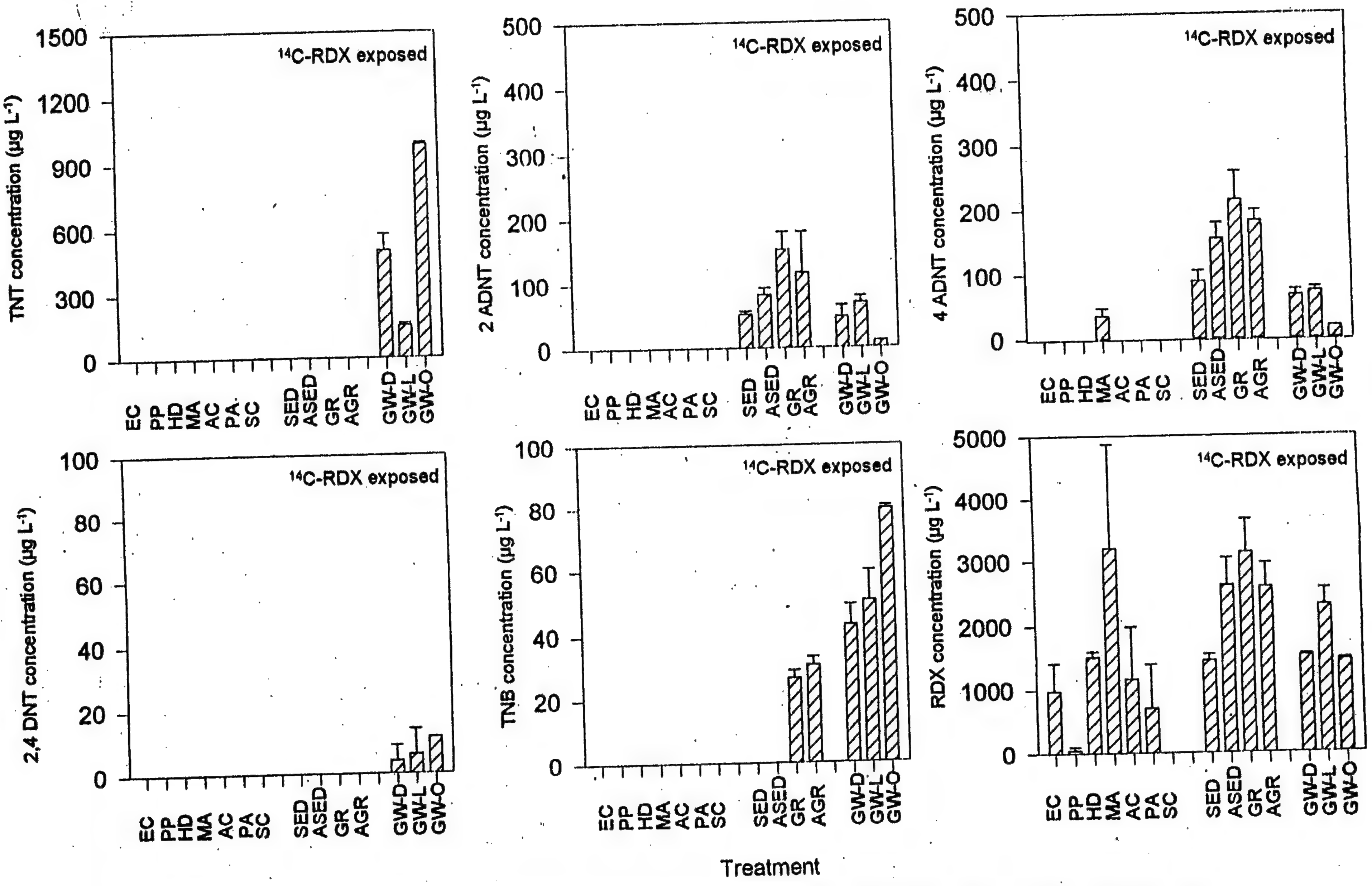

Figure 12. Explosives concentrations in [^{14}C]-RDX groundwater after 13-day incubation with plants, substrates, controls, and initially, as determined by HPLC analysis. Mean values and standard deviations (N=3). Abbreviations: EC, elodea; PP, pondweed; HD, water-stargrass; MA, parrot-feather; AC, sweet-flag; PA, reed canary grass; SC, wool-grass; SED, sediment; ASED, autoclaved sediment; GR, gravel; AGR, autoclaved gravel; GW-D, groundwater darkened; GW-L, groundwater illuminated; GW-O, initial groundwater.

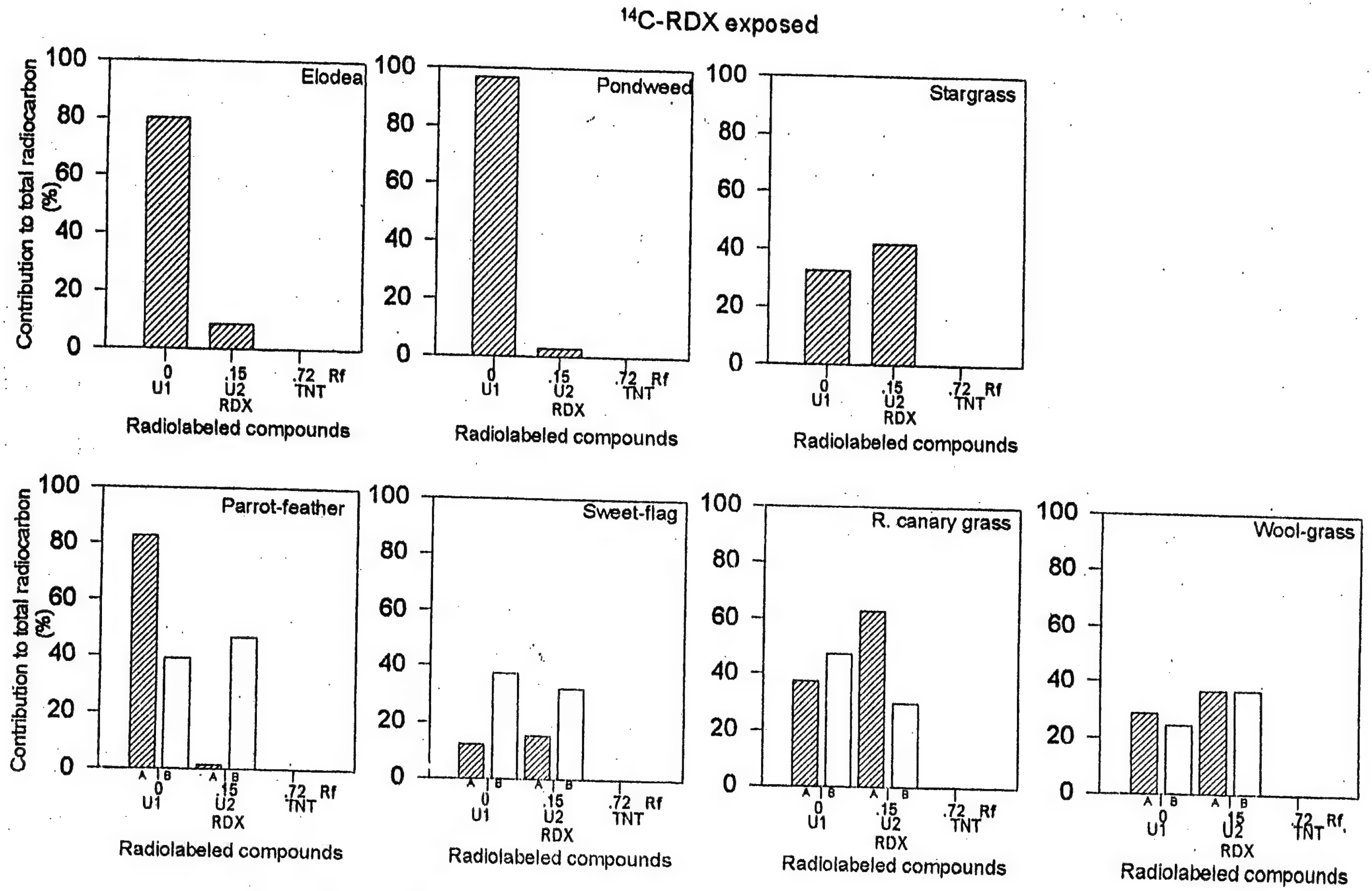

Figure 13. Distribution of RDX-derived radioactivity over acetonitrile extracted compounds from plants and substrates, expressed as percent of total counts per TLC lane. Separation by thin layer chromatography of extracts and references on Silica Gel 60F plates in a toluene:methanol mixture (98:2), using the Ambis Radioanalytic Imaging System for measuring radioactivity. Abbreviations: A, above-ground; B, below-ground; U, unknown.

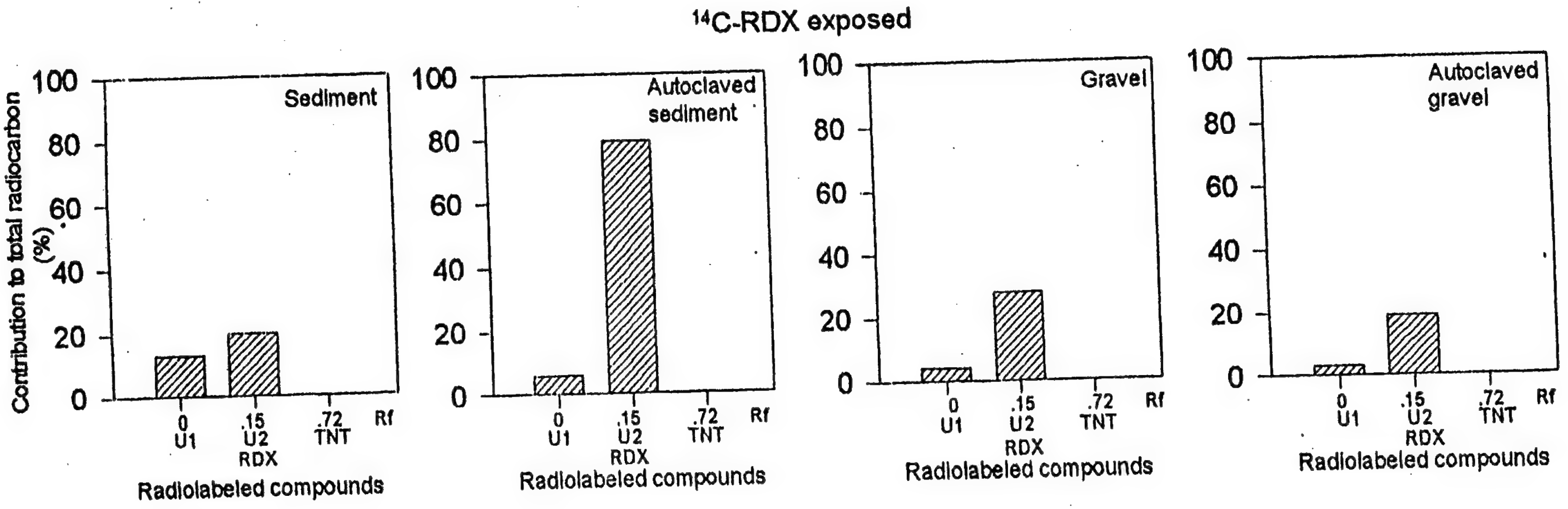

Figure 13- continued.

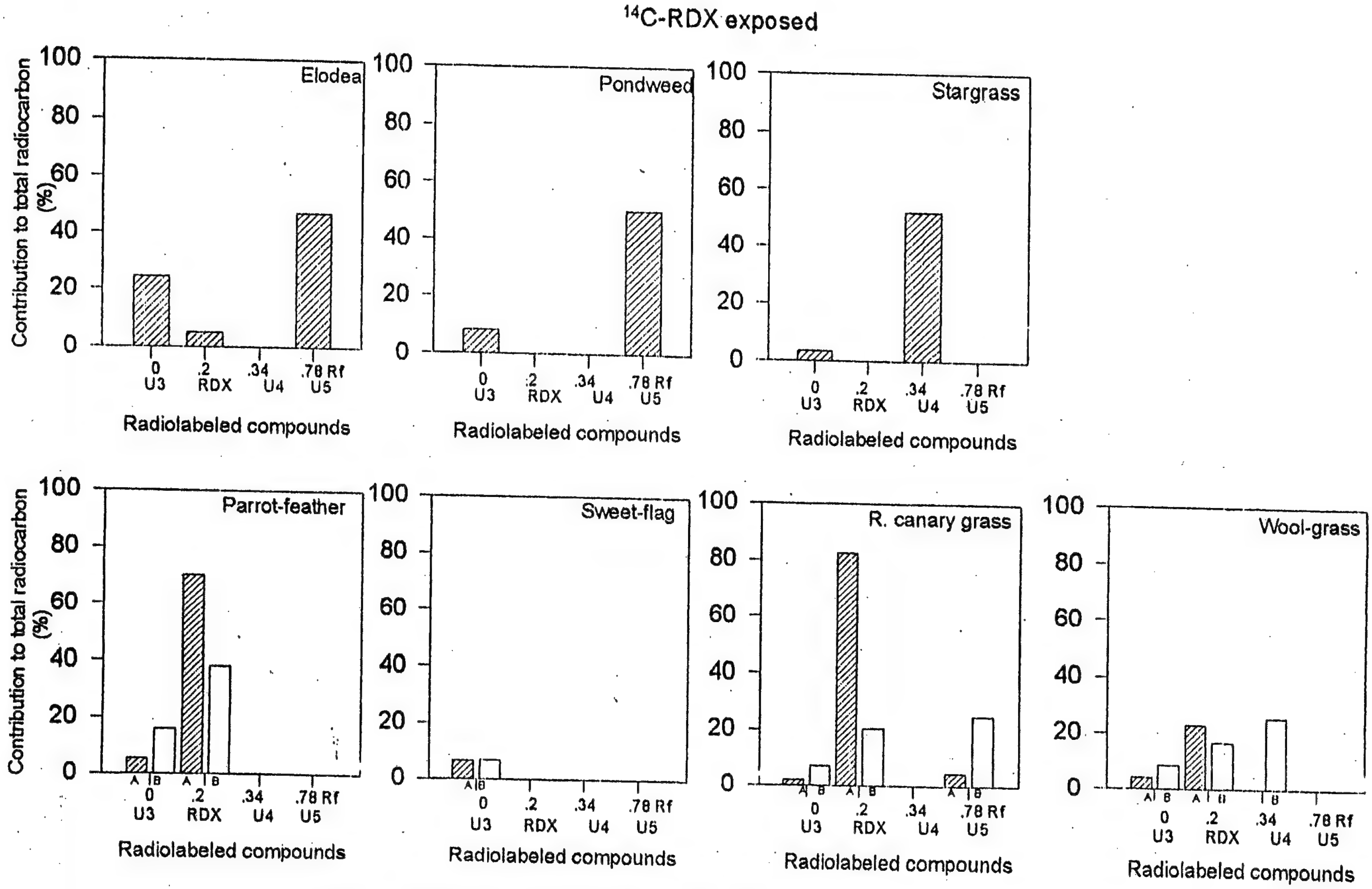

Figure 14. Distribution of RDX-derived radioactivity over acetonitrile extracted compounds from plants and substrates, expressed as percent of total counts per TLC lane. Separation by thin layer chromatography of extracts and references on Whatman Reversed Phase LKC18F plates in a water:methanol mixture (50:50), using the Ambis Radioanalytic Imaging System for measuring radioactivity. Abbreviations: A, above-ground; B, below-ground; U, unknown.

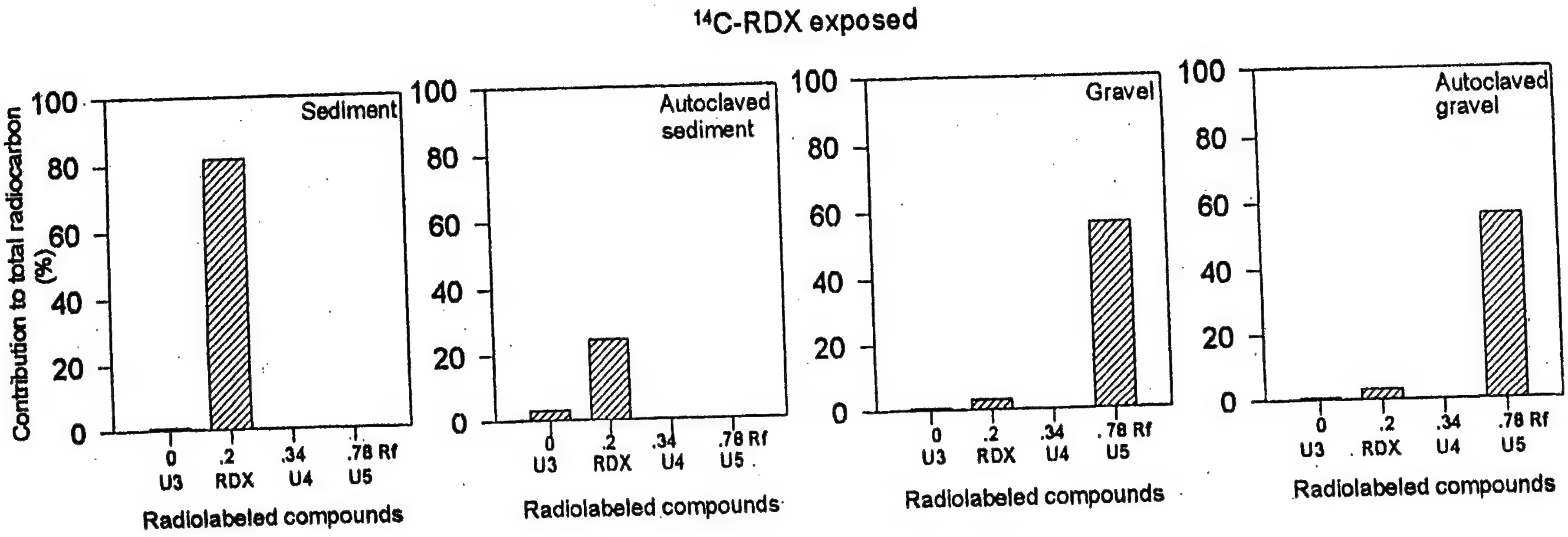

Figure 14- continued.

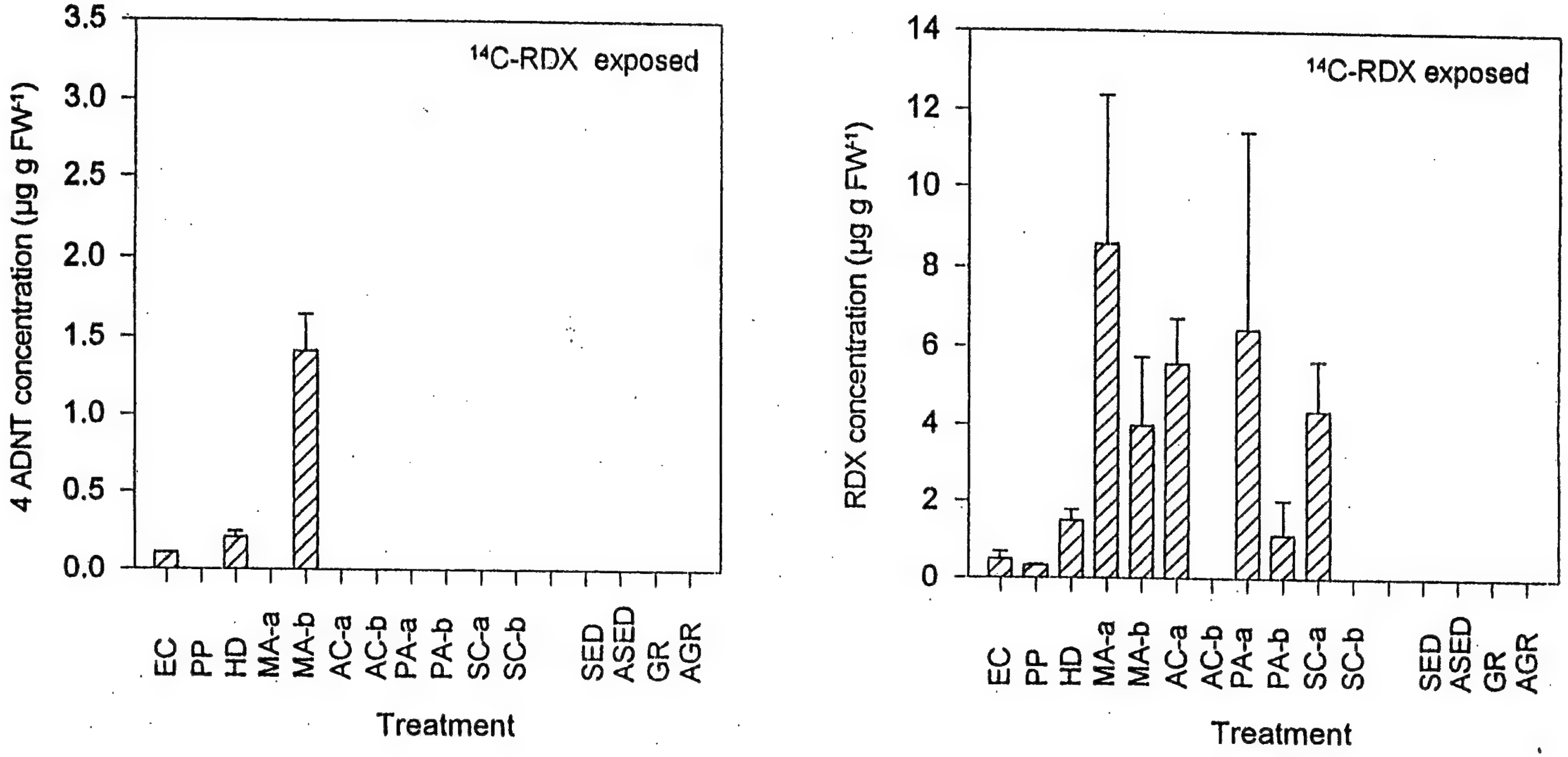

Figure 15. Explosives residues in plants and substrates after 13-day incubation in [^{14}C]-RDX groundwater, as determined by HPLC analysis. Mean values and standard deviations (N=3). Abbreviations: EC, elodea; PP, pondweed; HD, water-stargrass; MA, parrot-feather, -a above-ground and -b below-ground; AC, sweet-flag, -a above-ground and -b below-ground; PA, reed canary grass, -a above-ground and -b below-ground; SC, wool-grass, -a above-ground and -b below-ground; SED, sediment; ASED, autoclaved sediment; GR, gravel; AGR, autoclaved gravel.

Table 1. Aquatic and wetland plant species used to evaluate the behavior and fate of TNT and RDX in incubations with [^{14}C]-TNT or [^{14}C]-RDX amended groundwater from the Milan Army Ammunition Plant.

Group	Family	Plant species	
		Scientific name	Common name
Submersed			
Monocotyledons	Hydrocharitaceae	*Elodea canadensis* Rich. in Michx.	Elodea
	Potamogetonaceae	*Potamogeton pectinatus* L.	Sago pondweed
	Pontederiaceae	*Heteranthera dubia* (Jacq.) MacM.	Water star-grass
Emergent			
Dicotyledons	Haloragaceae	*Myriophyllum aquaticum* (Vell.) Verdc.	Parrot-feather
Monocotyledons	Araceae	*Acorus calamus* L.	Sweet-flag
	Gramineae	*Phalaris arundinacea* L.	Reed canary grass
	Cyperaceae	*Scirpus cyperinus* (L.) Kunth	Wool-grass

Note: U.S.Army Engineer Waterways Experiment Station, September-October 1996. Common names used in the text.

Table 2. Chemical characteristics of wetted Milan soil used in earlier screens for explosives removal (Best and Sprecher 1996). Soil of similar composition served as substrate in the Milan lagoons, from which the presently used sediment was sampled. Mean values ± s.d. (N=3).

Parameter	Concentration	Unit
Nitrogen	1.4659 ± 0.055	g kg DW^{-1}
Exchangeable NH_4-N	0.007 ± 0.000	g kg DW^{-1}
Phosphorus	0.447 ± 0.014	g kg DW^{-1}
Available PO_4-P	0.067 ± 0.002	g kg DW^{-1}
Bulk density	1.246 ± 0.009	g DW mL^{-1}
Moisture	269.1 ± 0.78	g H_2O kg FW^{-1}
Organic matter	39.6 ± 0.13	g kg DW^{-1}

Abbreviations: DW = dry weight; FW = fresh weight

Table 3. Chemical characteristics of the filtered groundwater from the Milan Army Ammunition Plant. pH and bicarbonate concentrations of this water were modified subsequent to these data before incubation. Mean values ± s.d. (N=3).

Characteristic	Value
pH	6.6 ± 0.1
Macro-, micro-nutrients (mg L^{-1})	
Alkalinity	20 ± 1
Kjeldahl-N	0.114 ± 0.161
NO_3-N	0.092 ± 0
NH_3-N	0.323 ± 0.009
Total-P	-
PO_4-P	0.0002 ± 0
SO_4	0.76 ± 0.03
Ca	4.7 ± 0.1
Fe	-
Explosives (µg L^{-1})	
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	92.7 ± 1.2
2,6-Diamino-,4-nitro-toluene (2,6DANT)	-
2,4-Diamino-,6-nitrotoluene (2,4DANT)	-
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	1443.3 ± 17.0
1,3,5-Trinitro-benzene (TNB)	79.4 ± 1.0
1,3-Dinitro-benzene (1,3DNB)	-
2, 4, 6-Trinitrotoluene (TNT)	988.0 ± 9.1
2-Amino-dinitrotoluene (2ADNT)	9.3 ± 0
4-Amino-, 2, 6-dinitrotoluene (4ADNT)	18.2 ± 0.2
2,4-Dinitrotoluene (2,4DNT)	11.4 ± 0
2,6-Dinitrotoluene (2,6DNT)	-
2, 2', 6, 6-Tetranitro- 4, 4-azoxytoluene	-

Note: - Below detection.

Table 4. Experimental design and initial fresh weights (mean values ± s.d.; N=3) per incubation type. Incubations were explosives-contaminated MAAP groundwater: 1) amended with [^{14}C]-TNT for 7 days and re-dosed with [^{14}C]-TNT plus unlabeled TNT halfway; and 2) amended with [^{14}C]-RDX for 13 days. Incubated groundwater volume was 0.8 L.

Treatment	Incubator	Beakers and dimensions			Illumination	Initial fresh weight (g FW)	
		Number	Height (cm)	Diameter (cm)		[^{14}C]-TNT incubation	[^{14}C]-RDX incubation
Plant species							
Submersed							
Elodea	1	3	25	9	+	10.44 ± 0.23	13.47 ± 0.21
Sago pondweed	1	3	25	9	+	11.51 ± 0.66	23.30 ± 0.62
Water star-grass	1	3	25	9	+	11.39 ± 0.92	21.67 ± 1.49
Emergent							
Parrot-feather	1	3	19	12	±	23.50 ± 0.98	41.33 ± 3.13
Sweet-flag	1	3	19	12	±	131.60 ± 0.33	50.27 ± 2.81
Reed canary grass	1	3	19	12	±	67.90 ± 9.06	17.43 ± 2.21
Woolgrass	1	3	19	12	±	164.00 ± 11.11	92.03 ± 6.50
Substrates							
Sediment	1	3	19	8	+	26.45 ± 0	26.45 ± 0
Autoclaved sediment	1	3	19	8	+	26.45 ± 0	26.45 ± 0
Gravel	1	3	19	8	+	20.05 ± 0	20.05 ± 0
Autoclaved gravel	1	3	19	8	+	20.05 ± 0	20.05 ± 0
Controls							
Groundwater	1	3	19	8	-		
Groundwater	1	3	19	8	+		

Table 5. Explosives concentrations and quantities initially present in MAAP groundwater, and added as analyte or as radiolabel for incubation.

Timing	TNT Concentration Incubation Water (µg L^{-1})	Volume Added (mL)	TNT Incubation Water (µg replicate^{-1})	Radiolabel Incubation Water (Bq replicate $^{-1}$)
[^{14}C]-TNT incubation				
1. Initial				
Groundwater	988	800	790	0
Added anal.-TNT	-	-	-	-
Added [^{14}C]-TNT		1	519	55.5 x 10^4
Total	1637		1309	55.5 x 10^4
2. At redosing				
Groundwater	NM*	NM	NM*	
Added anal.-TNT		1	2175	0
Added [^{14}C]-TNT		1	519	55.5 x 10^4
Total			2694	55.5 x 10^4
Overall exposure	5004		4003	111.0 x 10^4

Timing	RDX Concentration Incubation Water (µg L^{-1})	Volume Added (mL)	RDX Incubation Water (µg replicate^{-1})	Radiolabel Incubation Water (Bq replicate $^{-1}$)
[^{14}C]-RDX incubation				
1. Initial				
Groundwater	1443	800	1154	0
Added [^{14}C]-RDX		1	69	55.5 x 10^4
Total exposure	1529		1223	55.5 x 10^4

Abbreviation: NM, not measured.
* Presumed to be below detection.

Table 6. Distribution of radioactivity over plant organs or portions after 7-day incubation in [^{14}C]-TNT groundwater, as indicated by radioanalytic imaging. Relative intensity of labeling expressed as +.

Plant species	Organ or portion			
	Root	Stem	Leaf	New shoot
Submersed				
Elodea			+	
Pondweed	++	+	++	
Water star-grass	+	++	+++	
	Root	Lower shoot	Upper shoot	Apical shoot
Emergent				
Parrot-feather	++	+		+
Sweet-flag	+++	+		+
R.canary grass	++	+		
Wool-grass	+++			

Table 7. Initial+ redosed radioactivity and analyte TNT in [^{14}C]-TNT groundwater, and after 7-day incubation with plants, substrates, or controls. Mean radioactivity, analyte TNT, and TNT-equivalent removal rates were calculated from these values. Radioactivity and analyte TNT: mean values and s.d. between parentheses (relative % of mean; N=3). TNT-equivalents: mean values.

Treatment	Initial + redosed		After 7-day incubation			Mean removal rate		
	DPM x 10^6 per repl.	mg TNT per repl.	DPM x 10^6 per repl.	^{14}C recovery (% total added)	mg TNT per repl.	DPM x 10^6 repl.$^{-1}$ d^{-1}	mg TNT repl.$^{-1}$ d^{-1}	TNT-equiv. mg repl.$^{-1}$ d^{-1}
Plant species								
Submersed								
Elodea	57.5 (14%)	4.003*	34.3 (14%)	52.0 (7%)	-	3.3 (14%)	—	0.230
Pondweed	70.5 (10%)	4.003*	35.0 (4%)	53.0 (2%)	-	5.1 (18%)	—	0.290
Water star-grass	68.5 (0%)	4.003*	33.5 (9%)	50.8 (4%)	-	4.7 (4%)	—	0.275
Emergent								
Parrot-feather	70.3 (2%)	4.003*	29.5 (10%)	44.7 (4%)	-	5.8 (6%)	—	0.330
Sweet-flag	71.3 (1%)	4.003*	5.8 (19%)	8.8 (2%)	-	9.4 (2%)	—	0.528
R.canary grass	67.3 (2%)	4.003*	5.6 (26%)	8.5 (2%)	-	8.8 (1%)	—	0.523
Wool-grass	64.4 (2%)	4.003*	4.6 (39%)	7.0 (2%)	-	9.3 (5%)	—	0.578
Substrates								
Sediment	70.7 (0%)	4.003*	39.8 (6%)	60.3 (3%)	0.140 (38%)	4.4 (7%)	0.552 (1%)	0.249
Autocl.sediment	71.3 (2%)	4.003*	42.3 (5%)	64.1 (3%)	0.645 (23%)	4.1 (2%)	0.480 (3%)	0.230
Gravel	72.7 (3%)	4.003*	47.4 (3%)	71.8 (2%)	0.021 (29%)	3.6 (11%)	0.569 (0%)	0.198
Autocl.gravel	70.5 (2%)	4.003*	49.7 (3%)	75.3 (2%)	0.813 (8%)	3.0 (13%)	0.456 (1%)	0.170
Controls								
Groundwater/D	65.0 (1%)	4.003*	68.0 (1%)	103.0 (1%)	3.127 (10%)	NA	0.125 (16%)	NA
Groundwater/L	68.7 (3%)	4.003*	66.8 (6%)	101.2 (6%)	2.408 (6%)	NA	0.228 (6%)	NA

Abbreviations: repl., replicate; D dark; L light; NA, not applicable.

*, Initial concentration 1.637 mg TNT L^{-1}; redosed with 3.367 mg TNT L^{-1} after 3 days; initial incubation volume 0.8 L.

-, below detection.

—, Removal was 100% at the end of incubation; therefore per day rate not calculated.

Table 8. Removal rates of radioactivity and TNT-equivalents from [^{14}C]-TNT groundwater over 7-day incubation with plants, substrates, or controls. Removal rates on basis of initial mass. Radioactivity: mean values and s.d. between parentheses (relative % of mean; N=3).TNT-equivalents: mean values.

Treatment	Removal rate			
	DPM x 10^6 g total DW^{-1} d^{-1}	TNT-equiv. mg g total DW^{-1} d^{-1}	DPM x 10^6 g below-gr.DW^{-1} d^{-1}	TNT-equiv. mg g below-gr.DW^{-1} d^{-1}
Plant species				
Submersed				
Elodea	4.985 (14%)	0.347		
Pondweed	5.549 (21%)	0.315		
Water star-grass	8.785 (11%)	0.513		
Emergent				
Parrot-feather	1.150 (2%)	0.065	3.154 (2%)	0.180
Sweet-flag	0.309 (2%)	0.056	0.454 (2%)	0.025
Reed canary grass	0.617 (14%)	0.059	1.065 (14%)	0.063
Wool-grass	0.199 (4%)	0.012	0.415 (4%)	0.026
Substrates				
Sediment	0.266 (7%)	0.015		
Autoclaved sediment	0.250 (2%)	0.014		
Gravel	0.191 (11%)	0.011		
Autoclaved gravel	0.156 (13%)	0.009		
Controls				
Groundwater/Dark	NA	NA		
Groundwater/Light	NA	NA		

Abbreviations: below-gr., below-ground; NA, not applicable

Table 9. Mass balances for [^{14}C]-TNT-derived radioactivity in 7-day incubations of groundwater with plants, substrates, or controls. Radioactivity in plants and substrates determined by combustion. Compartment contributions in percent of total radioactivity added (mean values and s.d. between parentheses; N=3).

Treatment	Groundwater		Aerial CO_2-C*	Volatile organic C*	Carbon in plants or substrates	Recovery
	Total C	[(HCO_3^- + CO_2)-C]				
Plant species						
Submersed						
Elodea	51.97 (7)	[5.27 (4)]	0.09	0.01	23.91 (4)	75.98
Pondweed	52.96 (2)	[0]	0.07	0.01	58.39 (5)	111.43
Water star-grass	50.77 (4)	[2.09 (1)]	0.07	0	79.46 (19)	130.30
Emergent						
Parrot-feather	44.68 (4)	[0]	0.04	0.01	73.13 (19)	117.86
Sweet-flag	8.80 (2)	[0.45 (0)]	0.15	0.01	83.39 (14)	92.35
R.canary grass	8.43 (2)	[0.87 (0)]	0.07	0	51.23 (41)	59.73
Wool-grass	7.00 (3)	[0.05 (0)]	0.06	0	87.36 (5)	94.42
Substrates						
Sediment	60.30 (3)	[5.15 (2)]	0.14	0.09	35.42 (14)	96.95
Autocl.sediment	63.94 (3)	[4.16 (6)]	0.11	0.13	39.80 (34)	103.98
Gravel	71.89 (2)	[10.06 (5)]	0.13	0.06	28.18 (3)	100.26
Autocl.gravel	75.35 (2)	[5.81 (3)]	0.08	0.13	21.98 (3)	97.54
Controls						
Groundwater/D	103.07 (1)	[0]	0.04	0		103.11
Groundwater/L	101.26 (6)	[4.70 (5)]	0.05	0.16		101.47

Abbreviations: D dark; L light; conc., concentration.

*, Single value. **, S.d. between parentheses (relative % of mean; N=3). Condensation was always <0.01%.

[ ], not included in balance.

Table 10. Distribution of radioactivity in plants or substrates after 7-day incubation in [^{14}C]-TNT groundwater. Total radioactivity was determined by combustion, extractable radioactivity by extraction with acetonitrile and LS. Radioactivity per g and total radioactivity in mass: mean values in DPM x 10^6 and s.d. between parentheses (relative % of mean; N=3). Final mass: mean values ± s.d. (N=3). Extractable radioactivity, in 1) mean values in DPM x 10^6 and s.d. between parentheses (relative % of mean; N=3), and 2) relative as % of total radioactivity in mass determined by combustion and LS (mean values ± s.d.; N=3).

Species or substrates	Radioactivity per g (DPM x 10^6 g FW^{-1})	Final plant or substrate mass (g FW)	Total radioactivity in mass (DPM x 10^6)	Extractable radioactivity from plants or substrates	
				(DPM x 10^6 g FW^{-1})	(% total radio-activity mass)
Plant species					
Submersed					
Elodea	1.83 (24%)	8.36 ± 0.77	15.78 (18%)	0.309 (28%)	18.04 ± 6.15
Pondweed	2.88 (9%)	8.02 ± 0.02	38.54 (9%)	0.473 (13%)	16.68 ± 3.19
Water star-grass	2.78 (9%)	11.22 ± 1.62	52.45 (24%)	0.685 (7%)	24.91 ± 2.18
Emergent					
Parrot-feather/a	0.03 (25%)	36.97 ± 6.18	0.80 (30%)	0.006 (20%)	21.67 ± 1.69
————— /b	1.61 (34%)	19.37 ± 3.71	47.47 (26%)	0.136 (26%)	8.79 ± 1.93
Sweet-flag/a	0.02 (78%)	48.90 ± 6.21	0.82 (88%)	0.003 (59%)	22.92 ± 4.09
———— /b	0.72 (30%)	82.83 ± 11.11	54.22 (16%)	0.114 (53%)	15.04 ± 3.40
R.canary grass/a	0.01 (57%)	11.63 ± 8.35	0.18 (67%)	0.001 (19%)	17.73 ± 10.47
——————/b	0.89 (96%)	31.33 ± 23.27	33.62 (81%)	0.143 (36%)	33.24 ± 20.00
Wool-grass/a	0.01 (8%)	49.03 ± 2.46	0.29 (12%)	0.001 (28%)	12.82 ± 3.18
—————/b	0.75 (29%)	90.60 ± 14.20	95.04 (14%)	0.065 (41%)	8.37 ± 1.76
Substrates					
Sediment	0.42 (40%)	60.26 ± 5.63	23.38 (42%)	0.042 (34%)	9.92 ± 2.14
Autocl. sediment	0.36 (83%)	79.50 ± 6.94	26.27 (88%)	0.588 (44%)	464 ± 428
Gravel	0.26 (%)	70.76 ± 1.31	18.60 (11%)	0.057 (10%)	22.64 ± 4.68
Autocl. gravel	0.22 (%)	65.00 ± 0.75	14.51 (12%)	0.378 (19%)	176 ± 31

Abbreviations: /a, above-ground; /b, below-ground.

Table 11. Distribution of [^{14}C]-TNT-derived radioactivity over acetonitrile extracted compounds from plants and substrates, expressed as percent of total counts per TLC lane. Separation by TLC of extracts and references on Silica Gel 60F plates in a toluene:methanol mixture (98:2), using the Ambis Radioanalytic Imaging System for measuring radioactivity. Recovery: radioactivity counted in spots relative to that counted per lane, as percent.

Species or substrates	Rf-value							Reco-very (%)	Radio-label lane^{-1} (counts)	Front (cm)
	0 U1	0.09 U2	0.19 ADNTs	0.62 TNT	0.78 U3	0.91 U4	0.93 U5			
Plant species										
Submersed										
Elodea	84.3		3.4					87.7	18536	16.2
Pondweed	84.2		4.2					88.4	28482	16.2
Water star-grass	68.7				4.9			73.6	13066	16.2
Emergent										
Parrot-feather/a	30.8							30.8	1414	16.2
—————/b	82.6		6.6					89.2	12231	16.2
Sweet-flag/a	39.7							39.7	1515	16.2
—————/b	83.1							83.1	8590	16.2
R.canary grass/a	14.1							14.1	1755	16.2
—————/b	46.7		7.1	4.9			29.0	87.7	19829	16.2
Wool-grass/a	9.6					21.9		31.5	1478	16.2
—————/b	76.0							76.0	6472	16.2
Substrates										
Sediment	48.0							48.0	2977	16.2
Autocl.sediment	53.7	2.7	15.7	11.0				83.1	12755	16.2
Gravel	53.1	6.8	7.8	8.7				76.4	8583	16.2
Autocl.gravel	11.0	7.7		17.2				35.9	8380	16.2

Abbreviations: /a, above-ground; /b, below-ground; U, unknown.

Table 12. Distribution of radioactivity over plant organs or portions after 13-day incubation in [^{14}C]-RDX-labeled groundwater, as indicated by radioanalytic imaging. Relative intensity of labeling expressed as +.

Plant species	Organ or portion			
	Root	Stem	Leaf	New shoot
Submersed				
Elodea				+++
Pondweed	++	+	+	+++
Water star-grass	+	+	+	
	Root	Lower shoot	Upper shoot	Apical shoot
Emergent				
Parrot-feather	+	+	++	+++
Sweet-flag	+			++
R.canary grass	++	+		+++
Wool-grass	+	+		++

Table 13. Initial radioactivity and analyte RDX in [^{14}C]-RDX groundwater, and after 13-day incubation with plans, substrates, or controls. Mean radioactivity, analyte RDX, and RDX-equivalent removal rates were calculated from these values. Radioactivity and analyte-RDX: mean values and s.d. between parentheses (relative % of mean; N=3). RDX-equivalents: mean values.

Treatment	Initial		After 13-day incubation			Mean removal rate		
	DPM x 10^6 per repl.	mg RDX per repl.	DPM x 10^6 per repl.	^{14}C recovery (% total added)	mg RDX per repl.	DPM x 10^6 repl.$^{-1}$ d^{-1}	mg RDX repl.$^{-1}$ d^{-1}	RDX-equiv. mg repl.$^{-1}$ d^{-1}
Plant species								
Submersed								
Elodea	33.9 (0%)	1.223*	20.8 (12%)	63.0 (7%)	0.568 (42%)	1.0 (19%)	0.050 (37%)	0.036
Pondweed	33.8 (1%)	1.223*	7.2 (43%)	21.8 (9%)	0.024 (141%)	2.0 (14%)	0.092 (3%)	0.072
Water star-grass	33.5 (2%)	1.223*	24.7 (7%)	74.8 (5%)	0.875 (6%)	0.7 (12%)	0.027 (15%)	0.026
Emergent								
Parrot-feather	34.8 (6%)	1.223*	20.9 (25%)	63.3 (6%)	0.725 (25%)	1.1 (45%)	0.038 (37%)	0.039
Sweet-flag	35.8 (1%)	1.223*	16.7 (41%)	50.6 (21%)	0.455 (73%)	1.5 (37%)	0.059 (44%)	0.051
R.canary grass	35.9 (1%)	1.223*	18.7 (19%)	56.7 (1%)	0.319 (93%)	1.3 (22%)	0.070 (33%)	0.044
Wool-grass	39.2 (11%)	1.223*	3.7 (42%)	11.2 (4%)	-	2.7 (11%)	0.094 (0%)	0.084
Substrates								
Sediment	34.4 (1%)	1.223*	22.5 (8%)	68.2 (5%)	0.583 (8%)	0.9 (17%)	0.049 (8%)	0.032
Autocl.sediment	34.2 (0%)	1.223*	24.0 (6%)	72.7 (5%)	0.798 (5%)	0.8 (16%)	0.032 (10%)	0.029
Gravel	34.3 (0%)	1.223*	31.5 (0%)	95.5 (0%)	1.131 (1%)	0.2 (5%)	0.007 (18%)	0.007
Autocl.gravel	34.2 (0%)	1.223*	31.1 (0%)	94.2 (0%)	1.104 (1%)	0.2 (3%)	0.008 (8%)	0.007
Controls								
Groundwater/D	33.1 (5%)	1.223*	33.8 (5%)	102.4 (5%)	1.172 (0%)	NA	0.004 (10%)	NA
Groundwater/L	33.6 (1%)	1.223*	34.5 (0%)	104.5 (0%)	1.165 (1%)	NA	0.004 (16%)	NA

Abbreviations: repl., replicate; D dark; L light; NA, not applicable.

*, Initial concentration 1.529 mg RDX L^{-1}; initial incubation volume 0.8 L -, below detection;

Table 14. Removal rates of radioactivity, analyte RDX, and RDX-equivalents from [^{14}C]-RDX groundwater over 13-day incubation with plants, substrates, or controls. Removal rates on basis of initial mass. Radioactivity and analyte-RDX: mean values and s.d. between parentheses (relative % of mean; N=3). RDX-equivalents: mean values.

Treatment	Removal rate					
	DPM x 10^6 g tot.DW^{-1} d^{-1}	mg RDX g tot.DW^{-1} d^{-1}	RDX-equiv. mg g tot.DW^{-1}d^{-1}	DPM x 10^6. g below-gr. DW^{-1} d^{-1}	mg RDX g below-gr. DW^{-1} d^{-1}	RDX-equiv. mg g below-gr. DW^{-1}d^{-1}
Plant species						
Submersed						
Elodea	1.175 (19%)	0.058 (36%)	0.042			
Pondweed	0.794 (12%)	0.035 (2%)	0.029			
Water star-grass	0.491 (7%)	0.019 (9%)	0.018			
Emergent						
Parrot-feather	0.123 (50%)	0.004 (43%)	0.004	0.338 (50%)	0.012 (43%)	0.012
Sweet-flag	0.130 (43%)	0.005 (50%)	0.004	0.192 (43%)	0.008 (50%)	0.007
R.canary grass	0.359 (23%)	0.019 (36%)	0.012	0.619 (23%)	0.032 (36%)	0.021
Wool-grass	0.104 (5%)	0.004 (7%)	0.002	0.217 (5%)	0.007 (7%)	0.007
Substrates						
Sediment	0.055 (17%)	0.003 (8%)	0			
Autocl. sediment	0.047 (16%)	0.002 (10%)	0			
Gravel	0.011 (5%)	0.0003 (17%)	0			
Autocl. gravel	0.013 (3%)	0.00004 (84%)	0			
Controls						
Groundwater/D	NA	NA				
Groundwater/L	NA	NA				

Abbreviations: below-gr., below-ground; tot., total; D dark; L light; NA, not applicable

Table 15. Mass balances for [^{14}C]-RDX-derived radioactivity in 13-day incubations of groundwater with plants, substrates, or controls. Radioactivity in plants and substrates determined by combustion. Compartment contributions in percent of total radioactivity added (mean values and s.d. between parentheses; N=3.

Treatment	Groundwater		Aerial CO_2-C*	Volatile organic C*	Carbon in plants or substrates	Recovery
	Total C	[(HCO_3^- + CO_2)-C]				
Plant species						
Submersed						
Elodea	63.04 (7)	[1.27 (0)]	0.70	0.01	47.83 (8)	111.58
Pondweed	21.86 (9)	[4.19 (1)]	2.76	0.01	57.98 (13)	82.61
Water star-grass	74.76 (5)	[1.01 (1)]	0.97	0	18.31 (3)	94.04
Emergent						
Parrot-feather	63.25 (16)	[0.32 (0)]	1.02	0.01	20.52 (10)	84.80
Sweet-flag	50.47 (21)	[0.87 (1)]	4.06	0.01	20.60 (4)	75.14
R.canary grass	56.64 (11)	[1.30 (1)]	5.05	0.03	21.36 (7)	83.08
Wool-grass	11.22 (4)	[0.37 (0)]	10.17	0.03	35.01 (11)	56.43
Substrates						
Sediment	68.19 (5)	[7.94 (1)]	2.68	0.08	3.40 (2)	74.35
Autocl.sediment	72.66 (5)	[0.99 (0)]	1.03	0.11	3.45 (2)	77.25
Gravel	95.48 (0)	[1.61 (2)]	0.55	0.03	1.63 (0)	97.69
Autocl.gravel	94.24 (0)	[0]	0.91	0.05	1.60 (0)	96.80
Controls						
Groundwater/D	103.65 (1)	[0.59 (1)]	0.30	0		103.95
Groundwater/L	104.65 (0)	[0]	0.30	0.02		105.47

Abbreviations: D dark; L light; conc., concentration.

* Single value. ** S.d. between parentheses (relative % of mean; N=3). Condensation was always <0.01%.

[ ], not included in balance.

Table 16. Distribution of radioactivity in plants or substrates after 13-day incubation in $[^{14}C]$-RDX groundwater. Total radioactivity was determined by combustion, extractable radioactivity by extraction with acetonitrile and LS. Radioactivity per g and total radioactivity in mass: mean values in DPM x 10^6 and s.d. between parentheses (relative % of mean; N=3). Final mass: mean values ± s.d. (N=3). Extractable radioactivity, in 1) mean values in DPM x 10^6 and s.d. between parentheses (relative % of mean; N=3), and 2) relative as % of total radioactivity in mass determined by combustion (mean values ± s.d.; N=3).

Species or substrates	Radioactivity per g (DPM x 10^6 g FW^{-1})	Final plant or substrate mass (g FW)	Total radioactivity in mass (DPM x 10^6)	Extractable radioactivity from plants or substrates	
				(DPM x 10^6 g FW^{-1})	(% total radio-activity mass)
Plant species					
Submersed					
Elodea	0.419 (24%)	17.75 ± 0.53	8.123 (21%)	0.100 (16%)	24.47 ± 3.26
Pondweed	0.628 (21%)	28.09 ± 0.82	19.135 (23%)	0.083 (3%)	13.96 ± 3.19
Water star-grass	0.246 (12%)	23.71 ± 2.12	6.041 (18%)	0.072 (10%)	29.58 ± 2.01
Emergent					
Parrot-feather/a	0.383 (49%)	21.16 ± 1.55	5.506 (54%)	0.239 (56%)	59.94 ± 5.19
--------------- /b	0.199 (37%)	8.78 ± 0.88	1.268 (31%)	0.122 (38%)	60.93 ± 4.88
Sweet-flag/a	0.211 (40%)	11.83 ± 2.36	1.562 (48%)	0.025 (60%)	10.55 ± 3.88
---------- /b	0.138 (12%)	35.33 ± 0.42	5.237 (13%)	0.050 (32%)	35.23 ± 7.67
R.canary grass/a	0.721 (12%)	5.50 ± 2.24	3.204 (41%)	0.205 (19%)	28.15 ± 1.95
----------------/b	0.231 (13%)	15.74 ± 2.92	3.845 (27%)	0.059 (25%)	26.90 ± 10.62
Wool-grass/a	0.341 (52%)	30.8 ± 3.83	8.797 (54%)	0.051 (34%)	16.82 ± 6.14
------------/b	0.106 (19%)	52.07 ± 2.58	7.275 (16%)	0.022 (29%)	20.70 ± 2.70
Substrates					
Sediment	0.017 (53%)	76.97 ± 5.12	1.124 (48%)	0.003 (47%)	24.79 ± 11.55
Autoclaved sediment	0.017 (51%)	72.93 ± 7.07	1.139 (61%)	0.011 (72%)	59.30 ± 13.62
Gravel	0.008 (4%)	63.60 ± 2.38	0.538 (10%)	0.005 (10%)	61.76 ± 10.26
Autoclaved gravel	0.008 (15%)	65.93 ± 1.20	0.527 (16%)	0.004 (6%)	57.69 ± 8.39

Abbreviations: /a, above-ground; /b., below-ground

Table 17. Distribution of [^{14}C]-RDX-derived radioactivity over acetonitrile extracted compounds from plants and substrates, expressed as percent of total counts per TLC lane. Separation by TLC of extracts and references on Silica Gel 60F plates in a toluene:methanol mixture (98:2), using the Ambis Radioanalytic Imaging System for measuring radioactivity. Recovery: radioactivity counted in spots relative to that counted per lane, as percent.

Species or substrates	Rf-value			Recovery (%)	Radio-label lane^{-1} (counts)	Front (cm)
	0 U1	0.15 U2/RDX	0.72 TNT			
Plant species						
Submersed						
Elodea	80.3	8.7		89.0	10095	17.0
Pondweed	96.7	3.0		99.7	10359	17.0
Water star-grass	32.6	41.9		74.5	6354	17.0
Emergent						
Parrot-feather/a	82.6	1.5		84.1	6166	17.0
---------------/b	38.9	46.8		85.7	8431	17.0
Sweet-flag/a	12.3	15.3		27.6	1425	17.0
-----------/b	37.1	32.1		69.2	5486	17.0
R.canary grass/a	37.2	62.8		100.0	40903	17.0
---------------/b	47.1	29.8		76.9	5381	17.0
Wool-grass/a	28.6	36.7		65.3	3704	17.0
-------------/b	24.5	36.7		61.2	3422	17.0
Substrates						
Sediment	12.8	19.9		32.7	1429	17.0
Autocl.sediment	5.9	79.5		85.4	8304	17.0
Gravel	4.2	28.0		32.2	1389	17.0
Autocl.gravel	3.0	19.1		22.1	1784	17.0

Abbreviations: /a, above-ground; /b, below-ground; Unknown.

Table 18. Distribution of [^{14}C]-RDX-derived radioactivity over acetonitrile extracted compounds from plants and substrates, expressed as percent of total counts per TLC lane. Separation by TLC of extracts and references on Whatman Reversed Phase LKC18F plates in a water:methanol mixture (50:50), using the Ambis Radioanalytic Imaging System for measuring radioactivity. Recovery: radioactivity counted in spots relative to that counted per lane, as percent.

Species or substrates	Rf-value				Recovery (%)	Radio-label lane^{-1} (counts)	Front (cm)
	0 U3	0.20 RDX	0.34 U4	0.78 U5			
Plant species							
Submersed							
Elodea	24.3	5.2		47.1	76.6	8403	10.2
Pondweed	8.5			50.2	58.7	5005	10.2
Water star-grass	3.5		52.1		55.6	4920	10.2
Emergent							
Parrot-feather/a	5.3	70.4			75.7	11930	10.2
----------------/b	15.8	38.0			53.8	7175	10.2
Sweet-flag/a	6.4				6.4	1413	10.2
-------------/b	6.7				6.7	2727	10.2
R.canary grass/a	1.9	82.4		4.4	88.7	30184	10.2
-------------------/b	7.0	20.4		25.0	52.4	5544	10.2
Wool-grass/a	3.9	22.8			26.7	3316	10.2
--------------/b	8.4	16.9	25.7		51.0	3267	10.2
Substrates							
Sediment	20.5				20.5	1390	10.2
Autocl.sediment	0.6	81.6			82.2	7711	10.2
Gravel	3.2	24.6			27.8	1103	10.2
Autocl.gravel	0.7	3.2		56.6	60.4	6582	10.2

Abbreviations: /a, above-ground; /b, below-ground; U, unknown.

Appendix A

Chemical Characteristics Milan Army Ammunition Plant Groundwater Before Filtration

Table. Chemical characteristics of unfiltered and untreated groundwater from the Milan Army Ammunition Plant (Well M-146) measured on 11 September 1996 by the Tennessee Valley Authority.

Characteristic	Value
pH	5.8
Macro-, micronutrients (mg L^{-1})	
Kjeldahl-N	0.37
NO_3-N	6.0
NH_3-N	0.32
PO_4-P	0.01
Ca	4.5
Fe	0.03
Explosives (µg L^{-1})	
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	106
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	1980
1,3,5-Trinitro-benzene (TNB)	109
2, 4, 6-Trinitrotoluene (TNT)	1359
2-Amino-dinitrotoluene (2ADNT)	-
4-Amino-, 2, 6-dinitrotoluene (4ADNT)	21.3
2,4-Dinitrotoluene (2,4DNT)	23

-, Below detection

Appendix B
Tables Supporting Figures

Appendix B- Table 1. Relative growth rates of plants over 7-day incubation in [^{14}C]-TNT groundwater. Mean values ± s.d. (N=3).

Plant species	Relative growth rate (g DW g DW^{-1} d^{-1})
Submersed	
Elodea	-0.0323 ± 0.0123
Pondweed	-0.0514 ± 0.0079
Water star-grass	0.0074 ± 0.0280
Emergent	
Parrot-feather	0.1516 ± 0.0152
Sweet-flag	0.0003 ± 0.0077
Reed canary grass	-0.0001 ± 0.0108
Wool-grass	-0.0237 ± 0.0027

Appendix B - Table 2. Evapotranspiration rates in [^{14}C]-TNT groundwater over 7-day incubation with plants, substrates, or controls. Mean values ± s.d. (N=3). Initial incubation volume 0.8 L.

Treatment	Evapotranspiration rate	
	mL replicate^{-1} d^{-1}	mL g above-ground DW^{-1} d^{-1}
Plant species		
Submersed		
Elodea	13.81 ± 0.67	
Pondweed	11.90 ± 2.69	
Water star-grass	12.38 ± 2.36	
Emergent		
Parrot-feather	36.90 ± 7.38	11.38 ± 16.08
Sweet-flag	55.48 ± 0.89	5.72 ± 0.08
Reed canary grass	39.29 ± 2.10	6.48 ± 0.57
Wool-grass	55.00 ± 5.25	2.27 ± 0.11
Substrates		
Sediment	28.57 ± 0.58	
Autoclaved sediment	31.67 ± 3.97	
Gravel	29.76 ± 5.29	
Autoclaved gravel	28.57 ± 5.18	
Controls		
Groundwater/Dark	2.38 ± 0.34	
Groundwater/Light	21.62 ± 2.15	

Appendix B - Table 3. Explosives concentrations in [^{14}C]-TNT groundwater, initially and after 7-day incubation with plants, substrates, or controls. 24DNT (11 µg L^{-1}) and TNB (79 µg L^{-1}) were only present in the initial groundwater. Mean values ± s.d. (N=3).

Treatment	Explosives concentration (µg L^{-1})					
	TNT	2ADNT	4ADNT	24DANT	26DANT	RDX
Initial						
Groundwater	988* ± 9	9 ± 0	18 ± 0	-	-	1443 ± 17
Plant species						
Submersed						
Elodea	-	-	37 ± 2	1074 ± 206	74 ± 10	-
Pondweed	-	-	58 ± 45	478 ± 31	37 ± 5	-
Water star-grass	-	103 ± 12	572 ± 51	134 ± 142	26 ± 29	1296 ± 143
Emergent						
Parrot-feather	-	-	257 ± 44	-	-	1906 ± 132
Sweet-flag	-	-	196 ± 78	-	-	2220 ± 370
R.canary grass	-	-	30 ± 13	-	-	-
Wool-grass	-	-	89 ± 50	-	-	339 ± 74
Substrates						
Sediment	234 ± 88	400 ± 42	542 ± 40	72 ± 33	20 ± 4	1346 ± 69
Autocl.sediment	1130 ± 317	289 ± 31	382 ± 20	-	-	1456 ± 76
Gravel	36 ± 13	334 ± 34	657 ± 68	20 ± 0	-	1653 ± 162
Autocl.gravel	1366 ± 191	264 ± 5	503 ± 11	-	-	1623 ± 111
Controls						
Groundwater/D	3993 ± 417	85 ± 3	113 ± 9	-	-	1630 ± 142
Groundwater/L	3716 ± 282	96 ± 7	128 ± 13	-	-	1593 ± 97

Abbreviations: D dark; L light.
-, below detection. * Total (initial + redosed) TNT concentration was 5004 µg L^{-1}.

Appendix B - Table 4. Explosives residues in plants and substrates after 7-day incubation in $[^{14}C]$-TNT groundwater. Mean values ± s.d. (N=3).

Treatment	Explosives concentration ($\mu g\ g\ FW^{-1}$)		
	4ADNT	RDX	4,4-Azoxytoluene
Plant species			
Submersed			
Elodea	0.794 ± 0.169	-	-
Pondweed	1.190 ± 0.161	-	-
Water star-grass	2.613 ± 0.375	0.995 ± 0.097	0.248 ± 0.201
Emergent			
Parrot-feather/a	-	-	-
------------------/b	2.480 ± 0.759	1.980 ± 0.552	-
Sweet-flag/a	-	-	-
-------------/b	-	-	1.927 ± 0.438
R.canary grass/a	-	-	-
------------------/b	0.688 ± 0.209	-	0.243 ± 0.344
Wool-grass/a	-	-	-
--------------/b	1.052 ± 0.467	-	-
Substrates			
Sediment	0.180 ± 0.031	0.197 ± 0.061	-
Autocl.sediment	0.311 ± 0.130	0.451 ± 0.159	-
Gravel	0.156 ± 0.030	0.167 ± 0.021	-
Autocl.gravel	0.292 ± 0.078	0.173 ± 0.036	-

Abbreviations: /a, above-ground; /b, below-ground
-, below detection.

Appendix B - Table 5. Relative growth rates of plants over 13-day incubation in [^{14}C]-RDX groundwater. Mean values ± s.d. (N=3).

Plant species	Relative growth rate (g DW g DW^{-1} d^{-1})
Submersed	
Elodea	0.0212 ± 0.0013
Pondweed	0.0144 ± 0.0007
Water star-grass	0.0068 ± 0.0050
Emergent	
Parrot-feather	-0.0197 ± 0.0058
Sweet-flag	-0.0022 ± 0.0011
Reed canary grass	0.0128 ± 0.0132
Wool-grass	-0.0072 ± 0.0026

Appendix B - Table 6. Evapotranspiration rates in [^{14}C]-RDX groundwater over 13-day incubation with plants, substrates, or controls. Mean values ± s.d. (N=3). Initial incubation volume 0.8 L.

Treatment	Evapotranspiration rate	
	mL replicate^{-1} d^{-1}	mL g above-ground DW^{-1} d^{-1}
Plant species		
Submersed		
Elodea	16.15 ± 1.44	
Pondweed	14.49 ± 2.09	
Water star-grass	16.54 ± 0.94	
Emergent		
Parrot-feather	37.69 ± 12.26	6.78 ± 2.57
Sweet-flag	25.77 ± 8.99	6.87 ± 2.16
Reed canary grass	27.18 ± 4.41	17.21 ± 0.75
Wool-grass	40.64 ± 7.37	2.97 ± 0.39
Substrates		
Sediment	30.26 ± 3.98	
Autoclaved sediment	37.18 ± 5.03	
Gravel	32.82 ± 4.80	
Autoclaved gravel	28.08 ± 4.08	
Controls		
Groundwater/Dark	1.92 ± 0.54	
Groundwater/Light	22.05 ± 4.04	

Appendix B - Table 7. Explosives concentrations in $[^{14}C]$-RDX groundwater, initially and after 13-day incubation with plants, substrates, or controls. Mean values ± s.d. (N=3).

Treatment	Explosives concentration ($\mu g\ L^{-1}$)					
	TNT	2ADNT	4ADNT	24DNT	TNB	RDX
Initial						
Groundwater	988 ± 9	9 ± 0	18 ± 0	11 ± 0	79 ± 1	1443* ± 17
Plant species						
Submersed						
Elodea	-	-	-	-	-	976 ± 431
Pondweed	-	-	-	-	-	42 ± 59
Water star-grass	-	-	-	-	-	1496 ± 83
Emergent						
Parrot-feather	-	-	35 ± 12	-	-	3196 ± 1665
Sweet-flag	-	-	-	-	-	1156 ± 822
R.canary grass	-	-	-	-	-	704 ± 681
Wool-grass	-	-	-	-	-	-
Substrates						
Sediment	-	52 ± 6	89 ± 16	-	-	1443 ± 95
Autocl.sediment	-	82 ± 11	154 ± 25	-	-	2606 ± 427
Gravel	-	152 ± 27	215 ± 44	-	26 ± 2	3120 ± 530
Autocl.gravel	-	116 ± 63	182 ± 17	-	30 ± 3	2583 ± 365
Controls						
Groundwater/D	500 ± 75	48 ± 18	66 ± 9	4 ± 5	43 ± 6	1513 ± 19
Groundwater/L	148 ± 12	69 ± 10	73 ± 6	6 ± 8	50 ± 9	2296 ± 258

Abbreviations: D dark; L light.

-, below detection.* Total initial RDX concentration was 1529 $\mu g\ L^{-1}$.

Appendix B - Table 8. Explosives residues in plants or substrates after 13-day incubation in [^{14}C]-RDX groundwater. Mean values ± s.d. (N=3).

Treatment	Explosives concentration (µg g FW^{-1})		
	4ADNT	RDX	4,4-Azoxytoluene
Plant species			
Submersed			
Elodea	0.108 ± 0	0.481 ± 0.198	-
Pondweed	-	0.315 ± 0.036	-
Water star-grass	0.204 ± 0.039	1.470 ± 0.289	-
Emergent			
Parrot-feather/a	-	8.567 ± 3.777	-
---------------/b	1.407 ± 0.236	3.960 ± 1.757	-
Sweet-flag/a	-	5.560 ± 1.150	-
------------/b	-	-	-
R.canary grass/a	-	6.437 ± 4.985	-
---------------/b	-	1.117 ± 0.894	-
Wool-grass/a	-	4.350 ± 1.276	-
------------/b	-	-	-
Substrates			
Sediment	-	-	-
Autocl.sediment	-	0.299 ± 0.244	-
Gravel	-	0.165 ± 0.021	-
Autocl.gravel	-	0.134 ± 0.029	-

Abbreviations: /a, above-ground; /b, below-ground.
-, below detection.

Appendix C
Detection Levels for Explosives in Plants

Detection limits of explosives in plant material, calculated cf. EPA method 8330 (USEPA 1992).
Method Detection Level (MDL): 2.998 x SD, calculated for seven replicates.
Laboratory Reporting Limit (LRL): 10 x SD, calculated for seven replicates

Compound	Spiked conc. (mg L^{-1})	Recovery (%)	Explosives concentration (µg g FW^{-1})		
			Mean ± SD	MDL	LRL
Elodea					
TNT	0.750	71.39	0.803 ± 0.018	0.054	0.182
2ADNT*	0.750	61.03	0.687 ± 0.032	0.096	0.323
4ADNT*	0.750	21.01	0.236 ± 0.025	0.074	0.246
24DNT	0.750	80.99	0.911 ± 0.045	0.135	0.451
26DNT	0.750	85.28	0.959 ± 0.015	0.044	0.147
NB	0.750	64.90	0.730 ± 0.023	0.068	0.226
DNB	0.750	85.41	0.960 ± 0.040	0.121	0.405
TNB	0.750	61.01	0.686 ± 0.014	0.041	0.137
2NT	0.750	87.81	0.988 ± 0.044	0.132	0.440
3NT	0.750	91.79	1.033 ± 0.022	0.066	0.220
4NT	0.750	90.42	1.017 ± 0.012	0.036	0.119
RDX	0.750	133.41	1.501 ± 0.108	0.324	1.080
HMX	0.750	121.89	1.371 ± 0.054	0.161	0.538
Tetryl	0.750	18.46	0.208 ± 0.021	0.062	0.208
Sweet-flag/above-ground					
TNT	2.000	104.57	3.137 ± 0.270	0.810	2.703
2ADNT*	2.000	88.50	2.655 ± 0.101		
4ADNT*	2.000				
24DNT	2.000	158.14	4.744 ± 0.390	1.170	3.902
26DNT	2.000	85.93	2.578 ± 0.513	1.539	5.135
NB	2.000	83.357	2.501 ± 0.761	2.281	7.609
DNB	2.000	177.36	5.321 ± 0.596	1.786	5.958
TNB	2.000	246.71	7.401 ± 4.522	13.557	45.221
2NT	2.000	159.79	4.794 ± 1.656	4.965	16.560
3NT	2.000	88.64	2.659 ± 0.384	1.151	3.838
4NT	2.000	73.79	2.214 ± 0.257	0.771	2.573
RDX	2.000	403.21	12.096 ± 2.740	8.213	27.396
HMX	2.000	58.79	1.764 ± 0.586	1.757	5.860
Tetryl	2.000	77.29	2.320 ± 0.131	0.392	1.307

Appendix C - Table continued

Compound	Spiked conc. (mg L^{-1})	Recovery (%)	Explosives concentration (µg g FW^{-1})		
			Mean ± SD	MDL	LRL
Sweet-flag/below-ground					
TNT	2.000	105.29	3.159 ± 0.193	0.580	1.934
2ADNT*	2.000	98.50	2.955 ± 0.149		
4ADNT*	2.000				
24DNT	2.000	169.07	5.072 ± 0.493	1.478	4.931
26DNT	2.000	95.86	2.876 ± 0.390	1.169	3.899
NB	2.000	97.71	2.931 ± 0.901	2.700	9.007
DNB	2.000	174.71	5.241 ± 0.554	1.662	5.544
TNB	2.000	78.37	2.351 ± 0.298	0.892	2.977
2NT	2.000	163.93	4.918 ± 1.434	4.298	14.335
3NT	2.000	49.71	1.491 ± 1.123	3.366	11.228
4NT	2.000	65.143	1.954 ± 0.892	2.673	8.915
RDX	2.000	431.57	12.947 ± 2.239	6.711	22.385
HMX	2.000	23.34	0.700 ± 0.377	1.129	3.767
Tetryl	2.000	105.71	3.171 ± 0.786	2.357	7.860

Two gram fresh plant material, ground in liquid N_2, was spiked with an acetonitrile solution containing known concentrations of explosives, extracted with acetonitrile, and cleaned up using Florisil and neutral alumina as described in the 'Materials and Methods' section. Recovery and concentrations as determined using a C18 column; CN column determinations served to confirm compound identity.

*4ADNT and 2ADNT co-elute on C18 column; for elodea CN column-values, and for sweet-flag 2ADNT+4ADNT concentrations are given.

Concentration in mg explosive per L plant extract was converted to µg g FW^{-1} by multiplication with 0.005 x ½ x 1000; i.e., 5 mL extract volume, 2 g FW extracted.

Appendix D
Abbreviations

AC	sweet-flag
2ADNT	2-amino-4,6-dinitrotoluene
4ADNT	4-amino-2,6-dinitrotoluene
ADNTs	total monoamino-dinitrotoluenes (= 2ADNT, 4ADNT)
AGR	autoclaved gravel
ASED	autoclaved sediment
4,4-azoxytoluene	2,2', 6,6-tetranitro-4,4-azoxytolene
24DANT	2,4-diamino-6-nitrotoluene
26DANT	2,6-diamino-4-nitrotoluene
DNB	dinitrobenzene
1,3DNB	1,3-dinitrobenzene
1,4DNB	1,4-dinitrobenzene
DNT	dinitrotoluene
24DNT	2,4-dinitrotoluene
26DNT	2,6-dinitrotoluene
DOM	dissolved organic matter
DPM	desintegrations per minute
DW	dry weight
EC	elodea
FW	fresh weight
GR	gravel
GSH	γ-glutamyl-cysteinylglycine
GW-D	groundwater incubated in darkness
GW-L	groundwater incubated illuminated
GW-O	initial groundwater
HD	water star-grass
HPLC	high performance chromatography
HSD	honest dignificant difference
LS	liquid scintillation counting
MA	parrot-feather
MNX	mono-nitroso-derivative of RDX
NB	nitrobenzene
2NT	2-nitrotoluene
3NT	3-nitrotoluene
4NT	4-nitrotoluene
NT	nitrotoluene
PA	reed canary grass
PP	sago pondweed
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine
SC	wool-grass
SED	sediment
SPE	solid phase extraction
TLC	thin layer chromatography
TNB	trinitrobenzene
TNT	2,4,6-trinitritoluene
TNX	tri-nitroso-derivative of RDX
XAD	resin